EJM
Erasmus Journal of Medicine

December 2012 • vol 3 - no 1

Erasmus Journal of Medicine: independent scientific journal

Higher health insurance contribution for smokers?

Opinion

Antioxidant supplementation in schizophrenia patients

Systematic review

Editorial comment

The little man inside the little brain

Original contribution

Female genital cosmetic surgery

Colofon

Erasmus Journal of Medicine is a scientific magazine by and for students of Erasmus MC University Medical Center Rotterdam. It was initiated by the MFVR (the medical students' organization of Erasmus MC). The journal appears twice a year. It will be published on paper (2000 copies) and on the EJM website (www.erasmusjournalofmedicine.nl).

The main purpose of the journal is to stimulate Erasmus MC medical students to read and write about medical scientific subjects, early in their career, and to get acquainted with a professional review and publishing process, either as an author, a reviewer or as an editor.
A secondary purpose is to make the results of excellent student-driven research known to others.
The journal contains papers describing original research (Full articles), systematic reviews (Systematic Reviews), summaries of recently conducted studies (Extended abstracts), short descriptions of research projects looking for students to participate (Research Projects), opinion papers written by students (Opinions), editorial comments and letters to the editor.
Erasmus Journal of Medicine is funded by Erasmus MC, through an unrestricted grant.

Publisher
Erasmus MC University Medical Center Rotterdam.

Formatting
Ditems Media, Monnickendam

Printing
Verhagen Communicatie BV, Hooglanderveen

Correspondence
Erasmus Journal of Medicine
Petra Erkens, editorial assistant
Room Gk-658
Postbus 2040
3000 CA Rotterdam
E-mail: ejm@erasmusmc.nl

Website
www.erasmusjournalofmedicine.nl. Like us on www.facebook.com/erasmusjournalofmedicine.

Foreword

Open access and social media

When experts predict that new technologies will drastically change our lives within a few years that should, on its own, be reason enough to doubt it. New technological resources made available to us will at best manage to gain a place in everyday life that is not only determined by those resources, but to a great extent also by personal preferences and habits. However, the emergence of open access publishing and social media has had a major impact over the past decade. Open access has made scientific institutions more transparent, resulting in a democratizing effect on the way in which society views scientific research. The authority of an institution or a renowned researcher can no longer be taken for granted. The public wants to know all the ins and outs. Non-scientists deem themselves capable of understanding, assessing and criticizing research. As such, this is a good thing as scientific research is a part of society and public reflection can enrich research with new insights and ideas. This is acknowledged by scientists who intentionally seek to engage the public in a dialogue. For some time now, patients' organizations have been valued discussion partners in medical research because of their vast knowledge.

The recent cases of scientific fraud are most likely not symptoms of an increase in the number of cases of fraud in the academic world, but the effect of increasing transparency and a reduction in the ease with which the authority of scientific authorities and senior functionaries in academic organizations is taken for granted.

Social media is like open access. In the field of patient care, an increasing number of public lists of, for example, 'the best physicians' and 'the best hospitals' are being published. Recently it was suggested that online public complaints registers should be set up to increase transparency and quality. This is not surprising in a time when patients have shared their experiences on Twitter and Facebook before even leaving hospital. In education, Facebook also often gives a straightforward and therefore useful addition to the traditional educational evaluations.

As researchers, physicians and lecturers we need to learn how to use open access and social media to its full potential. First and foremost they are technological resources. In order to make them beneficial to the quality of scientific research, education and patient care, we must take on the challenge of further developing these media together will all the users and incorporating them into our work.

Prof. Huibert Pols, Dean and Vice-chairman of the Executive Board of Erasmus MC

Contents

Erasmus Journal Of Medicine

FOREWORD 4

EDITORIAL

New Section: Science in Practice 6
Erik Dieters en Ajda Rowshani

Perpetual change in a learning environment 6
Diederik Dippel

EDITORIAL COMMENT

The little man inside the little brain 7
Maarten Frens

Indications for a carriermediated transport of cortisol in HepG2 cells 7
Martin Hoogduijn en Ajda Rowshani

ORIGINAL CONTRIBUTION

Is transport of cortisol in liver cells carrier-mediated? 8
Kit-Yi Yam, Erica van den Akker, Elisabeth van Rossum, Alies van Mullem, Theo Visser

Liquid based vs. conventional cytology for evaluation of fine needle aspiration biopsies obtained by pulmonary physicians A pilot study 13
Marlene Mende, Annemarie van Leeuwen, Marleen Bakker, Coenraad Koegelenberg

SYSTEMATIC REVIEW

What is the role of the cerebellum in language? A systematic review 16
Subramanian Venkatesana

The association between non-adherence to immuno-suppressive agents and graft function in renal transplant patients. A systematic review 20
Marije Notenboom, Eva Schippers, Anne-Marlijn Versnel, Nienke Wolfhagen, Lilian Mohammad Zadeh, Emma Massey and Marry de Klerk

Natriuretic peptide guided therapy versus clinically guided therapy in patients with chronic heart failure A systematic review of randomized controlled trials 26
Bart van Tunen, Fei Fan Choi, Jin Ming Cheng

The Efficacy and Safety of the HCV protease inhibitors telaprevir and boceprevir. A systematic review 30
Iris van Blitterswijk, Merel Broers, Lisanne Lawant, Bart Rijnders

"Does Antioxidant Supplementation Improve Symptoms inSchizophrenia Patients?" A systematic review from Alis Heshmatollah and Madelon de Jong.

Relationship between advanced maternal age and the mode of delivery. A systematic review 34
Floor Smit, Hedwig van Wijk, Cornalijn Gouw Hans Duvekot

Adult versus Pediatric Renal Allograft in Children: a comparison based on GFR and graft survival. A systematic review 38
Meike van Amelsfort, Julia Kobben, Sophia Kronig, Rozemarijn van der Mooren, Stella Nieuwenburg, Karlien Cransberg

Does antioxidant supplementation improve symptomes in schizophrenia patients. A systematic review 42
Alis Heshmatollah, Madelon de Jong, Hemmo Drexhage

OPINIONS

Smokers should not cough up a higher health insurance contribution 46
Marianne Langhout

Female genital cosmetic surgery: about patient choice and protection 48
Juliette van Kalmthout

ABSTRACT

Colchicine in FMF: effects on fertility and pregnancy 50
Tim Both, Jan van Laar, Martin van Hagen, Femke Bonte-Mineur, Paul van Daele

INSTRUCTIONS FOR EJM AUTHORS

Instructions for EJM authors 51

Guidelines for the storyline of a scientific article 52

Advice to the reviewers of EJM 54

New Section: Science in Practice

The Erasmus Journal of Medicine aims to promote the conduct of scientific research by medical students of the Erasmus University. By introduction of a new section; Science in Practice, we want to extend our field of interest from the preclinical to the clinical phase of education and warmly encourage clinical trainees and interns to discuss clinical problems with their peers. This section can be used to address diagnostic and therapeutic questions and/or dilemmas using both clinical and basic science. Examples are case reports, clinical quiz and work reports.

Being a medical doctor is a life occupied with many different professional demands covering the broad spectrum of patient care, research and education. The ultimate goal is to apply knowledge obtained from research, to keep moving forward and extend your knowledge while practicing Medicine. This is the motivation to start a new section to address patient care related questions combing clinical and basic science. We especially target the students in their internships who are on longer rotations, because of the longer time available to pay attention on special cases and to discuss the possibility of writing for EJM from the beginning with their supervisors. Moreover, these rotations are in a field of medicine of their own choice. This makes these internships ideal to take a look beyond the routine clinical duties of an intern and dig a little deeper into the beautiful world of *evidence based medicine*. What should not be forgotten is that a publication always stands out on your curriculum vitae. *A Clinical Case Report*? The subject of the report can be any problem or interesting case you could encounter in a clinical setting.

Case reports should present a clinical dilemma and an explanation how it was solved. Tell us about the case presentation, medical history, examination, tests performed, management and eventual outcome. Of course the student is expected to discuss this case based on the available scientific literature, thus backing the decisions up with scientific evidence. A *Clinical Quiz* should present a short patient scenario and a related concrete question about the disease or condition, preferably accompanied by a clinical image. Provide 4 plausible treatment options or courses of actions, with one being the preferred answer for this question. Conclude with an elaboration on all the 4 possible answers and why the one is preferred, a diagnosis and a short discussion. *Clinical Pictures* alone are also welcome for submission, as long as the message obtained from the clinical picture is backed up by scientific evidence. For all described sections signed informed consent must be obtained from the patient. If you are interested, please search http://www.erasmusmc.nl/ejm for calls and more detailed information.

Erik Dieters
Student at Erasmus University Medical Center Rotterdam, the Netherlands
E-mail: E.Dieters@erasmusmc.nl
Ajda T. Rowshani, MD, PhD
Nephrologist-Clinical Immunologist
Department of Nephrology and Transplantation
Erasmus University Medical Center Rotterdam, the Netherlands
E-mail: T.Rowshani@erasmusmc.nl

Perpetual change in a learning environment

The Erasmus Journal of Medicine is in its third publication year. And we are changing. As we announced in the previous issue the editorial process has been refurbished. We now have a dedicated team of reviewers, mostly staff members of Erasmus MC and students. In pairs of one student and one staff member, they will review submitted papers and help you, student authors, to improve your work in order to meet the standards of Erasmus Journal of Medicine. The process is coordinated by a team of editors, again consisting of staff members and students of Erasmus MC. In the colophon you will find the names of all those who are involved in the editorial process.

Change always comes at a cost. The cost this time was a delay in processing the papers you submitted. The editorial board had difficulties in keeping up with the stream of information from authors and reviewers. The next change we therefore implemented was setting up a professional administrative system to aid the review process and keep track of all papers. The coming issues will certainly benefit from this change.

The Erasmus Journal of Medicine wants to offer a learning environment to all who are involved. The Journal offers a platform for students who want to become acquainted with scientific writing and reviewing process as an author. It also offers the opportunity to students who want to review incoming papers. This is probably the best way to learn what is needed for a successful submission. For this reason we have made extensive instructions for reviewers. These can be found in this journal and on the EJM website (www.erasmusjournalofmedicine.nl). From now on, the Erasmus Journal of Medicine will be open for submissions from all medical students, trying to acquaint themselves with scientific writing. This means that submission of papers is not longer restricted to medical students from Erasmus MC University Medical Center.

We will demand more from our authors. Papers have to be formatted as indicated in the instructions for authors. Often, authors will discover that a paper that was awarded a high grade by their teacher, will not automatically qualify for publication, because we make different demands with respect to appropriateness for a broad readership, methodology and readability.

The EJM is keeping up with its time. The last change we brought about is e-publication of abstracts of accepted papers on our Facebook page (www.facebook.com/erasmusjournalofmedicine). Abstracts can be viewed on the day of acceptance, and comments can be posted. Judging on the many ”likes” we get, this activity is well received.

As always, we want to thank everyone who has contributed to the journal: authors, reviewers, editors, publisher and last but not least, our readers. We hope that Erasmus Journal of Medicine continues to contribute to the outstanding learning environment of Erasmus MC University Medical Center.

Prof. dr Diederik Dippel, MD, PhD
neurologist (chairman of the editorial board)

The little man inside the little brain

The systematic review of Venkatesan in the current issue of the Erasmus Journal of Medicine explores the evidence for a cerebellar role in language function. This link between a traditionally motor structure and a cognitive function may not be obvious at first, but fits well in current views of motor and cognitive functioning.

Ever since Decartes, the study of the human mind has separated the "higher order" process from the "lower order" processes. Higher order (or "cognitive") processes include memory, language use, and executive functions such as decision making and planning. The lower order processes include motor skills, basic sensory processing, and many other aspects of behaviour not deemed sufficiently "cognitive".

For a long time there have been researchers who challenged this division (e.g. Sherrington in 1906), and it is obviously a somewhat arbitrary separation of the field. More recently, the distinction between high-order cognitive behaviour and low-order sensory-motor behaviour was challenged in a different direction. Parts of the brain, i.e. the cerebellum and basal ganglia, that were considered parts of the motor system turned out to be connected to non-motor, cognitive areas of the cerebral cortex in much the same way that they are connected to the motor areas. This lead to the suggestion that there are similarities between the processing required for motor behaviour and the processing required for cognitive behaviours.

Current motor control theories suggest that the cerebellum predicts the consequences of movement (i.e. it acts as a "forward model", an internal model of real movement). This is used to optimize motor control. As long as no unexpected events occur, movements can be performed even in the absence of sensory feedback. This process is extremely challenging in a computational sense, because the motor system has many degrees of freedom that all need to be controlled simultaneously, and in real time. In this view, it is maybe not surprising that the cerebellum contains more than 70% of all the neurons in the brain, despite its name ('little brain'), and its size (10% of total brain volume).

When the forward model is confronted with persistent errors in the motor output (e.g. due to fatigue), its predictions are obviously wrong, and its output must be adjusted. Modulation that facilitates consistent behaviour in changing conditions is called "adaptation." The adaptive process requires plasticity of the cerebellum..

The cerebellum receives extensive input from the neo-cortex. The cortical input is segregated, i.e. each cortical area sends input to a small number of cerebellar areas and these projections are largely non-overlapping. Each area in the cerebellum then projects back to its cortical input source, creating what have been called separate cortico-cerebellar loops. Cortical inputs are not restricted to classical movement-related areas in the cortex but also include many other areas, which are involved in language, planning and decision making.

Given the uniform structure of the cerebellum it is likely that all its regions function as a forward model that is, however, tailored to the processing in the cortical areas they are connected to. In this view, cerebellar regions connected to any part of the cortex predict the consequences of information processing in that cortical area. This perspective is supported by many experiments that have shown the cerebellum to be involved in non-motor function. Cognitive and motor skills are positively correlated in both young cerebellar patients and healthy controls. However, cognitive tests in cerebellar patients typically reveal only a mild phenotype, as do lesions of the cerebellum in primates, with the sole exception of cerebellar mutism, a condition where young patients after cerebellar surgery loose the ability to speak for a period of months. Hence, Venkatesans review of the link between cerebellum and language is both timely and important for the field.

Prof. Maarten A. Frens, PhD
Dept of Neuroscience, Erasmus MC University Medical Center
Rotterdam, the Netherlands
E-mail: m.frens@erasmusmc.nl

Indications for a carrier-mediated transport of cortisol in HepG2 cells

Glucocorticoids, such as the stress hormone called cortisol (hydrocortisone) in humans and corticosterone in rodents, belong to the group of steroid hormones that are secreted from the adrenal cortex in response to environmental or physiological stress. Dysregulated cortisol production may lead to disorders such as Cushing's disease or Addison's disease. Moreover, steroids are widely used as cornerstone drug in the treatment of many autoimmune diseases and also serve as a pivotal immunosuppressive drug in prevention of solid organ transplant rejection. Detailed knowledge of cortisol uptake by the cells will help us to understand drug delivery to the cell more precisely and find ways to adjust and to determine the dose that is really needed to execute the desirable therapeutic effects.This will help to prevent a broad range of side effects ranging from the development of diabetes to osteoporosis, to hypertension and muscle weakness.

In this paper 'Indications for a carrier-mediated transport of cortisol in HepG2 cells' Yam et al. set out to challenge the concept that cortisol uptake is merely mediated via passive diffusion. While the secretion of cortisol is tightly regulated via the hypothalamus-pituitary-adrenal axis, free diffusion of cortisol would render little control on the uptake of cortisol into cells. In an *in vitro* culture system using the hepatocarcinoma cell line HepG2, the authors show that the uptake of cortisol is independent of variations in pH and concentrations of the cortisol binding protein albumin. However, they demonstrate that cortisol uptake is temperature dependent and saturable at high extracellular cortisol concentrations, suggesting that the uptake of cortisol is an active process. By transfection of Hep2G and COS-1 cells with GR-α and OATP2B1, candidate transporter proteins for cortisol, it was attempted to increase the uptake of cortisol. Overexpression of these transporters did not result in increased uptake, demonstrating that these proteins are not involved in the transport of cortisol. Inhibition of the efflux transporter P-glycoprotein by tetracycline resulted in increased levels of intracellular cortisol. This suggests that the efflux of cortisol is at least to some extent dependent on a transport system. Other P-glycoprotein inhibitors did however not show this effect and bromosulfalein even significantly decreased the uptake of cortisol.

Altogether, the authors have collected an amount of data that suggests that a transport system is indeed present in HepG2 cells. This would provide a tool for pharmacological intervention in the regulation of intracellular cortisol levels. This type of research will eventually help defining patient tailored therapies and preventing overdosing and side effects on the long term.

Martin J. Hoogduijn PhD
Transplantation laboratory, Department of Internal Medicine
Erasmus University Medical Center Rotterdam, the Netherlands
E-mail: m.hoogduijn@erasmusmc.nl
Ajda T. Rowshani MD, PhD
Nephrologist-Clinical Immunologist
Department of Nephrology and Transplantation
Erasmus University Medical Center Rotterdam, the Netherlands
E-mail: T.Rowshani@erasmusmc.nl

Is transport of cortisol in liver cells carrier-mediated?

Kit-Yi Yam[a], Erica L.T. van den Akker[c], Elisabeth F.C. van Rossum[b], Alies A.A. van Mullem[b], Theo J. Visser[b]

[a] Biomedical Sciences student, Leiden University Medical Center, Leiden, The Netherlands
[b] Supervisor, Department of Internal Medicine, Section of Endocrinology, Erasmus University Medical Center, Rotterdam, The Netherlands
[c] Supervisor, Department of Pediatric Endocrinology, Erasmus MC-Sophia Children's Hospital, Rotterdam, The Netherlands
Correspondence: Kit-Yi Yam, e-mail: k.yam@umail.leidenuniv.nl

Cortisol is a steroid hormone that is thought to be taken up in a target cell by simple diffusion. The present study challenges this concept by characterizing cortisol transport in liver cells. In vitro transport studies with [3H]cortisol were carried out in HepG2 cells as a model for normal human liver cells. Various conditions were tested to optimize cellular cortisol uptake, including medium pH and protein concentration and overexpressing the glucocorticoid receptor (GR). The effects of incubation temperature and cortisol concentration were tested on both cortisol uptake and cortisol metabolism, and the effects of the efflux transporter inhibitors bromosulfalein, cyclosporin A, tetracycline and verapamil on cellular cortisol transport were determined. The results showed that cortisol uptake is a saturable process, suggesting carrier-mediated transport of cortisol. However, over-expression of GR or OATP2B1 did not affect cortisol uptake. HPLC analysis also demonstrated that cortisol was not metabolized in HepG2 cells. Additionally, efflux inhibitors did not show coherent effects. More studies should certainly be carried out, but the saturability of cellular cortisol uptake suggests that this is a transporter-mediated process.

Introduction

Natural glucocorticoids, such as the stress hormone cortisol in humans and corticosterone in rodents, belong to the group of steroid hormones that are secreted from the adrenal cortex in response to environmental or physiological stress [1]. The production of cortisol is regulated by the hypothalamus-pituitary-adrenal axis (HPA-axis) (Fig. 1). Certain stress signals, such as inflammation or pain, trigger the release of the corticotropin-releasing hormone (CRH) from the hypothalamus. CRH elicits the release of corticotropin (adrenocorticotropic hormone; ACTH) from the anterior pituitary gland. ACTH stimulates the synthesis and secretion of cortisol from the adrenal cortex. Cortisol has a negative feedback effect on the pituitary gland and the hypothalamus [2].

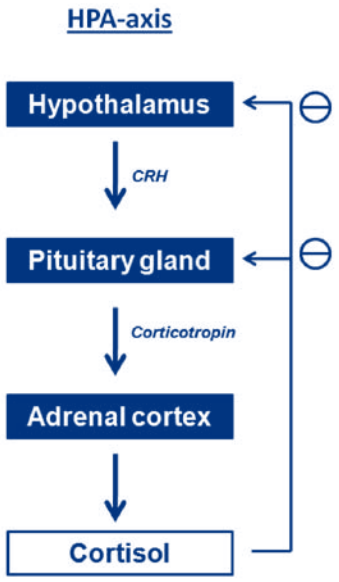


Figure 1 - Schematic overview of the HPA-axis
Regulation of the synthesis and secretion of cortisol by the HPA-axis is depicted. Thin black lines indicate the negative feedback of cortisol to the pituitary gland and the hypothalamus.
Adapted from Rhen T et al. N Engl J Med. 2005; 353: 1711-1723

Dysregulated cortisol production may lead to disorders such as Cushing's disease or Addison's disease. One characteristic of Cushing's disease is a high ACTH level, which may be caused by a pituitary gland tumor, resulting in an overproduction of cortisol. In contrast, patients with Addison's disease have decreased production of cortisol due to impaired steroidogenesis [1]. Cortisol plays an essential role in many processes such as regulating blood glucose levels, suppressing the immune system, stimulating gluconeogenesis in the liver and mobilizing amino acids as well as fatty acids [2].

In serum, approximately 95% of cortisol is bound to carrier proteins: 80-90% to cortisol-binding globulin (CBG) and 10-15% to albumin [3,4]. According to the free hormone hypothesis, steroid hormones are considered to be biologically inactive when bound to carrier proteins. Thus, the availability of free cortisol is determined by the amount of CBG and albumin that is present in serum [5].

The enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) catalyzes the inactivation of cortisol to cortisone. Conversely, 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), which is abundant in the liver, converts cortisone to cortisol [2,6]. The actions of glucocorticoids are mediated through binding to the major form of the glucocorticoid receptor (GR-α), which is a member of the steroid hormone receptor family. GR-β, which differs only in the last 15 amino acids from the GR-α isoform (777 amino acids), does not bind active glucocorticoids [7]. Binding of cortisol to GR-α results in the dissociation of molecular chaperones such as heat shock proteins (HSPs) (Fig. 2). GR functions as a hormone-activated nuclear transcription factor after it is phosphorylated. Thus, the cortisol-GR complex translocates to the nucleus, followed by binding to DNA target sequences called glucocorticoid-responsive elements (GREs), which leads to the transcription of target genes [2,6].

The effects of cortisol on different tissues are not only determined by the circulating cortisol concentrations and the activities of the 11β-HSD isoenzymes, they also depend on the transport of cortisol in and out of the cell.

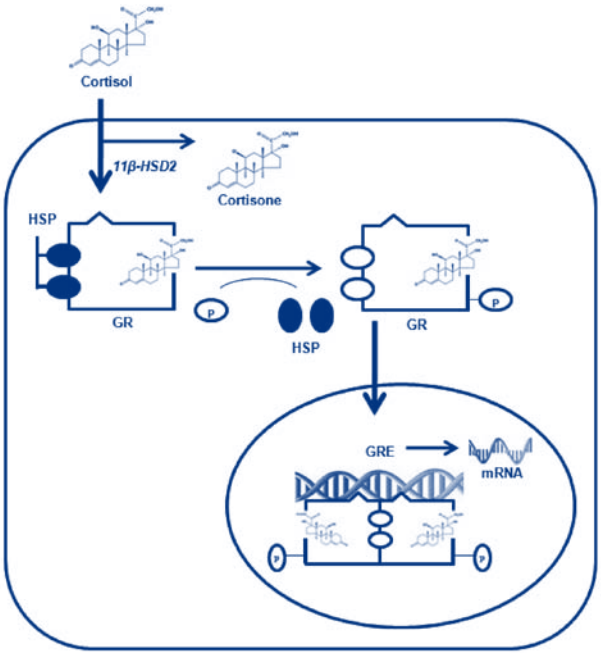


Figure 2 - Molecular mechanisms of glucocorticoid action
After binding to cortisol, the glucocorticoid receptor (GR) translocates to the nucleus. Following phosphorylation, heat shock proteins (HSPs) dissociate from GR, allowing active GR to bind to glucocorticoid-responsive elements (GREs). This process results in the transcription of target genes. 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) enzymes convert active cortisol to inactive cortisone. Adapted from Rhen T et al. N Engl J Med. 2005; 353: 1711-1723

Little is known about the nature of the transmembrane transport of cortisol although this is likely to be a key process in the tissue-specific regulation of cortisol action. Currently, no specific transporter is known for cortisol; it is therefore still assumed that this hormone moves through the plasma membrane by simple diffusion. This assumption is based on the fact that steroids are highly lipophilic substances that tend to cross through the lipid bilayer of the plasma membrane. However, previous studies have indicated that steroids cross the membrane not only by passive diffusion, but also by a transporter-mediated process [8]. Other studies have shown that the uptake of corticosterone is regulated by both non-saturable and saturable energy-dependent processes, demonstrating that inhibition of Na+/K+-ATPase results in decreased corticosterone uptake [9,10]. A study carried out in liver cells of rats demonstrated a higher cortisol uptake at 27°C when compared to a temperature of 5°C [11].

Similar to cortisol, it was initially postulated that thyroid hormone enters the cell by passive diffusion. Nevertheless, several transporters have been discovered that regulate the transport of thyroid hormones [12,13]. The organic cation transporter 3 (OCT3/SLC22A3) and organic anion transporting polypeptide 2 (OATP2/OATP1B1) are examples of transporters that show interactions with corticosterone. Studies carried out in rats demonstrate that serotonin transport by OCT3 can be inhibited by corticosterone, indicating the inhibition of uptake2-mediated monoamine clearance [14]. OATP2 is considered to play a role in the influx of [3H] cortisol as well as [3H]corticosterone at the choroid plexus and the pituitary gland [15].

Interaction also occurs between glucocorticoids and certain efflux transporters such as P-glycoprotein (P-gp; MDR1), which regulates the efflux of corticosterone and cortisol [15]. P-gp belongs to one of the three major human ABC transporters; others are breast cancer resistance protein (BCRP; ABCG2) and multidrug resistance-associated protein 2 (MRP2; ABCC2). Bromosulfalein (BSP) and tetracycline (TC) are specific inhibitors that inhibit MRP2 and P-gp, respectively. Other inhibitors, such as cyclosporin A (CSA) and verapamil (Ver), only block P-gp, BCRP or MRP2 partially (Table 1) [16].

Table 1 - Overview of inhibitors that specifically or partially inhibit efflux transporters

	Alternative nomenclature	BSP	CSA	TC	Ver
P-glycoprotein (P-gp)	ABCB1			* -	*
Breast cancer resistance protein (BCRP)	ABCG2		*		*
Multidrug resistance-associated protein 2 (MRP2)	ABCC2	* -	*		

P-gp, BCRP and MRP2 are efflux transporters that can be inhibited by BSP (bromosulfalein), CSA (cyclosporin A), TC (tetracycline) or Ver (verapamil). Underlined asterisks represent inhibitors that specifically block certain efflux transporters. Other asterisks represent inhibitors that are able to block more than one type of efflux transporter.
Adapted from Matsson P et al. Pharm Res. 2009; 26:1816-1831

The hypothesis of the present study was that the cellular uptake and efflux of cortisol is mediated not only by diffusion but also by transporters. To test this hypothesis, HepG2 cells were used to carry out in vitro transport studies with [3H]cortisol. Cortisol uptake was determined by incubating cells with the efflux inhibitors BSP, CSA, TC and Ver. Optimal conditions for cortisol uptake were determined by varying the pH value and protein (serum, albumin) concentration in the medium and by overexpressing GR-α. Variations in incubation temperature and different cortisol concentrations were also investigated. Cells were transfected with OATP2B1 to determine if uptake of cortisol is mediated by this transporter. High-Performance Liquid Chromatography (HPLC) analysis of medium and cell content were also used to determine cortisol metabolism.

Materials and methods

Cell culture
HepG2 (hepatocellular carcinoma) and COS-1 (SV40 transformed monkey kidney) cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM)/F-12+GlutaMAX supplemented with 9% heat inactivated fetal bovine serum (FBS) and 500 U/mL penicillin with 500 μg/mL streptomycin. Cells were incubated at 37°C under 5% CO2, maintained in 75 cm2 cell culture flasks and passaged when they reached 70-100% confluence.

Transport studies
COS-1 and HepG2 cells were seeded in 6-well plates and cultured at 37°C under 5% CO2 until they reached 70-100% confluence. Cortisol uptake was induced by incubating cells for different time periods with 10 nM, 10 μM or 100 μM cortisol (dissolved in ethanol) in incubation medium containing Dulbecco's phosphate buffered saline (DPBS) 0.1% D-glucose and 0.1% BSA, followed by addition of 0.5 μCi [1,2,6,7-3H]cortisol (79 Ci/mmol). Cortisol uptake was expressed as percentages of added cortisol.

Experiments were carried out using efflux inhibitors. BSP, TC and Ver were all dissolved in water and CSA in dimethyl sulfoxide (DMSO). Prior to the incubation with [3H]cortisol, cells were pre-incubated for 30 min with the inhibitors diluted in the incubation medium. The uptake of cortisol was controlled by adjusting the incubation medium to pH 5.3, 6.3 or 7.3 in combination with 0%, 0.1% or 1% BSA. In accordance with usual procedures, 0.1% BSA was added to prevent the adsorption of cortisol to plastic surfaces. Cells were also subjected to incubation medium containing 0%, 0.1% or 10% serum (in which CBG is present).

Incubation mixtures were brought to 25°C or 37°C before adding 1 mL to each well. Cells were washed before and after incubation with incubation medium. Afterwards, cells were lyzed with 500 μL 0.1 M NaOH and counted for radioactivity in a Packard Tri-Carb 2100 TR Liquid Scintillation Analyzer.

Transfection

HepG2 and COS-1 cells were transfected at a confluence of 70-90% for 48 h with 500 ng pcDNA3, pcDNA3.1.hGR-α or pCMV-SPORT6.OATP2B1 using X-tremeGENE 9, according to the manufacturer's protocol. Prior to the transfection, cells were refreshed with culture medium.

Cortisol metabolism

HPLC analysis was carried out with a Waters Alliance 2690, including Waters 960 Photodiode Array Detector. HepG2 cells (70-100% confluence) were cultured in 75 cm2 cell culture flasks and incubated for 1, 4 or 24 h with 10 mL incubation medium and 1 μM cortisol. Complete incubation medium was collected after the incubation; cells were washed with 10 mL incubation medium and lyzed with 6 mL 0.1 M NaOH. Medium and lysates were extracted with 1 mL ethylacetate. After evaporation of the solvent the residue was dissolved in 175 μL acetonitrile and mixed with 175 μL water. Samples (100 μL) were injected and the analytes were eluted on a reverse-phase Atlantis column. Absorbance of cortisol and possible metabolites was monitored at 254 nm.

Statistical analysis

All statistical analysis and graphics were performed by GraphPad Prism 5.0 software. The results of the cortisol uptake experiments are the means of duplicate determinations from two representative experiments. Values are expressed as the mean plus or minus SEM. P values were calculated using the student's t test and considered significant if $p<0.05$.

Suppliers

- Corning Inc., Corning, NY: 75 cm2 cell culture flasks
- Invitrogen, Breda, The Netherlands: Dulbecco's Modified Eagle's Medium/F-12+GlutaMAX, Dulbecco's Phosphate Buffered Saline, fetal bovine serum and pcDNA3
- Merck, Darmstadt, Germany: ethylacetate, acetonitrile
- Meriden, CT: Packard Tri-Carb 2100 TR Liquid Scintillation Analyzer, Quenchset from the Packard Instrument Company
- Open Biosystems, Huntsville, AL: pCMV-SPORT6.OATP2B1
- Perkin Elmer, Waltham, MA: [1,2,6,7-3H]cortisol (79 Ci/mmol), Picofluor
- Santa Cruz Biotechnology, Santa Cruz, CA: Cyclosporin A
- Sigma-Aldrich, Zwijndrecht, the Netherlands: cortisol, bromosulfalein, tetracycline, verapamil, bovine serum albumin, D-glucose, 500 U/mL penicillin, 500 μg/mL streptomycin, ethanol
- Waters, Etten-Leur, the Netherlands: Waters Alliance 2690, including Waters 960 Photodiode Array Detector, HPLC total recovery vials, Reverse-phase Atlantis column
- pcDNA3.1.hGR-α was a generous gift from Jan W. Koper (Dept. of Internal Medicine, Erasmus MC, Rotterdam, the Netherlands).
- Cell lines were obtained from Sigma-Aldrich or the American Type Culture Collection (ATCC-LGC, Wesel, Germany)

Results

Optimal conditions for cortisol uptake

Variations in pH of the incubation medium, the concentration of BSA or FBS in the medium and the cellular expression of GR-α were investigated to determine optimal conditions for uptake

Figure 3 - Effect of pH and BSA concentration on cortisol uptake by HepG2 cells. Incubation medium containing 0.1% BSA with a pH value of 7.3 tends to show optimum cortisol uptake. HepG2 cells were incubated for 15 min at 37°C with 10 nM cortisol.

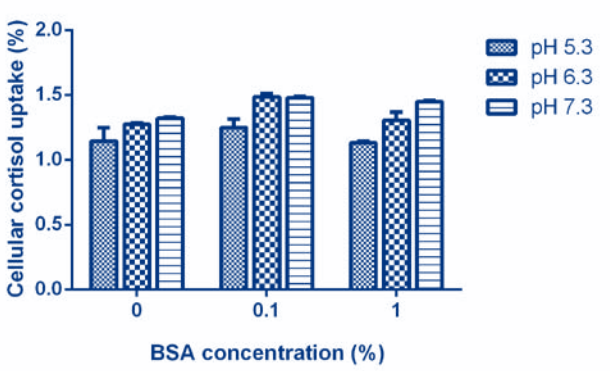
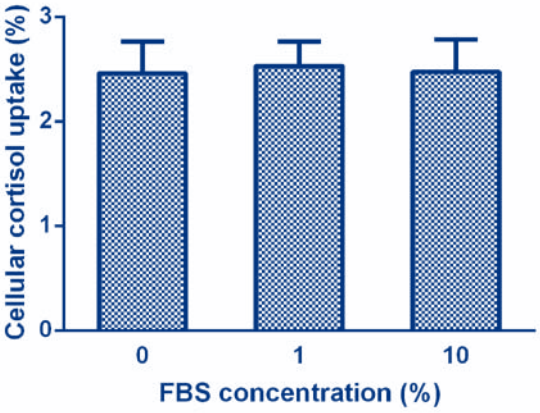


Figure 4 - Lack of effect of serum on cortisol uptake by HepG2 cells. Incubation of HepG2 cells for 10 min at 37°C with 10 nM cortisol in the presence of 0%, 1% or 10% FBS did not result in differences in cortisol uptake.



of cortisol (10 nM) by HepG2 cells. Also, possible transport of cortisol by the candidate transporter OATP2B1 was tested. In experiments carried out at pH 5.3, 6.3 and 7.3 with BSA concentrations of 0, 0.1 or 1%, optimal cortisol uptake was observed at 0.1% BSA and pH 7.3 (Fig. 3). However, differences with results obtained at other pH values and BSA concentrations were small. Cortisol uptake by HepG2 cells was also tested at pH 7.3 in the presence 0%, 1% or 10% FBS in the medium. Increasing the FBS concentration to 10% had no effect on cortisol uptake (Fig. 4). All further experiments were done at pH 7.3 in the presence of 0.1% BSA.

Transfection of HepG2 cells with GR-α did not change cortisol uptake (Fig. 5A). Also, transfection of COS-1 cells with the transporter candidate OATP2B1 did not increase cortisol uptake compared with control cells transfected with empty vector (pcDNA3) (Fig. 5B).

Uptake of cortisol is a saturable and temperature-dependent process

HepG2 cells were incubated for different time periods at 37 C with 10 nM, 10 μM or 100 μM cortisol (Fig. 6A). Irrespective of substrate concentration, cortisol uptake was rapid, and maximum uptake was reached after 2 min. However, uptake decreased with higher cortisol concentrations, suggesting that this process is saturable. Surprisingly, cortisol uptake by HepG2 cells was found to be higher when the incubation temperature was decreased from 37 to 25°C (Fig. 6B).

Figure 5 - Lack of effect of GR-α or OATP2B1 over-expression on cortisol uptake. Transfection of HepG2 cells with GR-α (A) of COS-1 cells with OATP2B1 (B) does not affect cortisol uptake during incubation of cells for 5 or 15 min at 37°C with 10 nM cortisol.

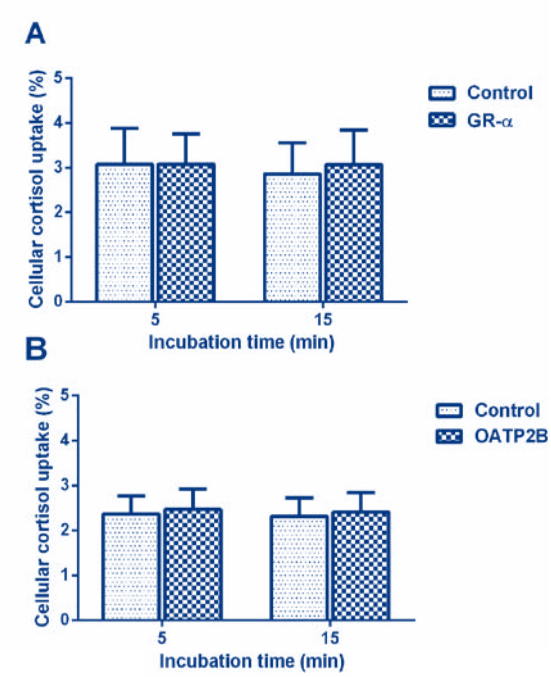
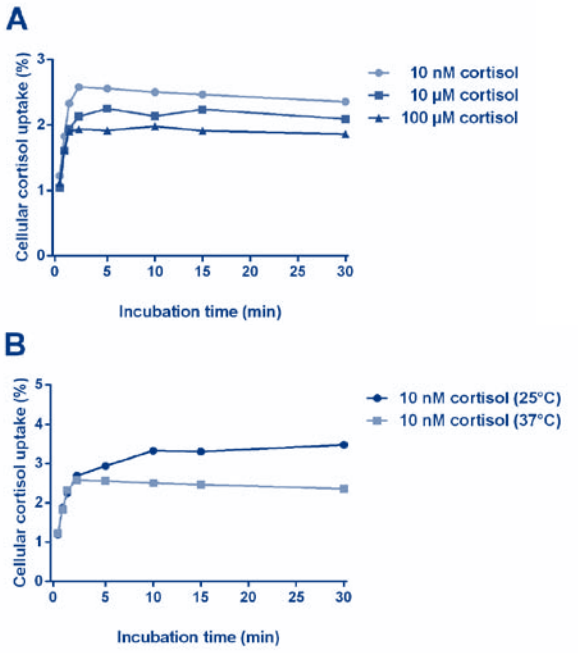


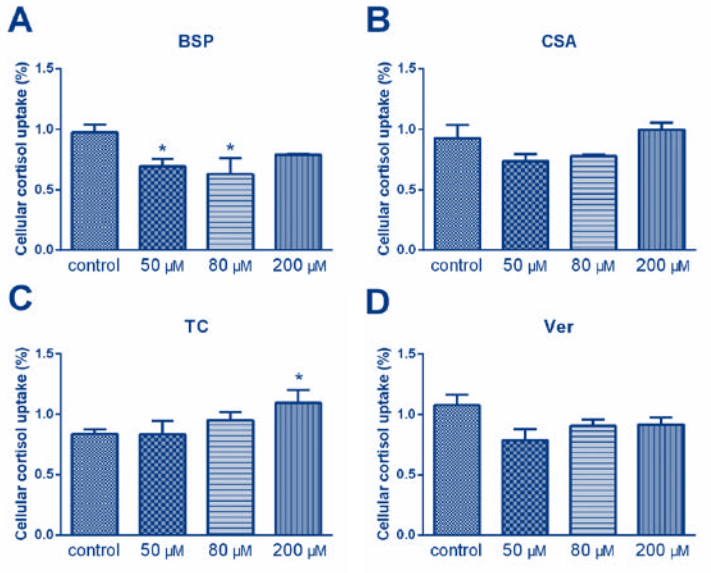
Figure 6 - Cortisol uptake in HepG2 cells is saturable and temperature dependent. (A) Maximum uptake was observed after incubation of HepG2 cells for 2 min at 37°C with 10 nM cortisol. The percentage of cortisol uptake was lower at the higher cortisol concentration of 100 µM, which indicates that this process is saturable. (B) Cortisol uptake was higher at an incubation temperature of 25°C compared to 37°C, which was observed at incubation times >2 min.



Influence of efflux inhibitors on cortisol uptake

The efflux inhibitors BSP, CSA, TC and Ver were tested on cortisol uptake in HepG2 cells. CSA and Ver did not produce significant differences in cortisol uptake (Fig. 7B,D). BSP at 50 μM and 80 μM significantly inhibited the uptake of cortisol (Fig. 7A), whereas TC at 200 μM significantly increased cortisol uptake (Fig. 7C).

Figure 7 - Effect of efflux inhibitors on cortisol uptake in HepG2 cells. (A) BSP significantly inhibited cortisol uptake at 50 µM and 80 µM. (B) Cortisol uptake was not significantly affected by CSA. (C) TC increased cortisol uptake, which was significant at a concentration of 200 µM. (D) Ver induced a small, insignificant decrease in cortisol uptake. All incubations were carried for 10 min at 37°C with 10 nM cortisol. *p<0.05 relative to the control.



Cortisol is not converted to cortisone in HepG2 cells

HPLC analysis was carried out with medium samples and cell lysates after incubation of HepG2 cells for 1, 4 and 24 h with 1 μM cortisol. The retention times of cortisone and cortisol were 23 and 25 min, respectively. Medium samples did not show any generation of cortisone after the different incubation periods (Fig. 8A-D). However, the cortisol peak showed a time-dependent decrease, suggesting metabolism of cortisol to other, unidentified products. These results indicate that cortisol metabolism is negligible during the short incubations to determine cellular cortisol uptake.

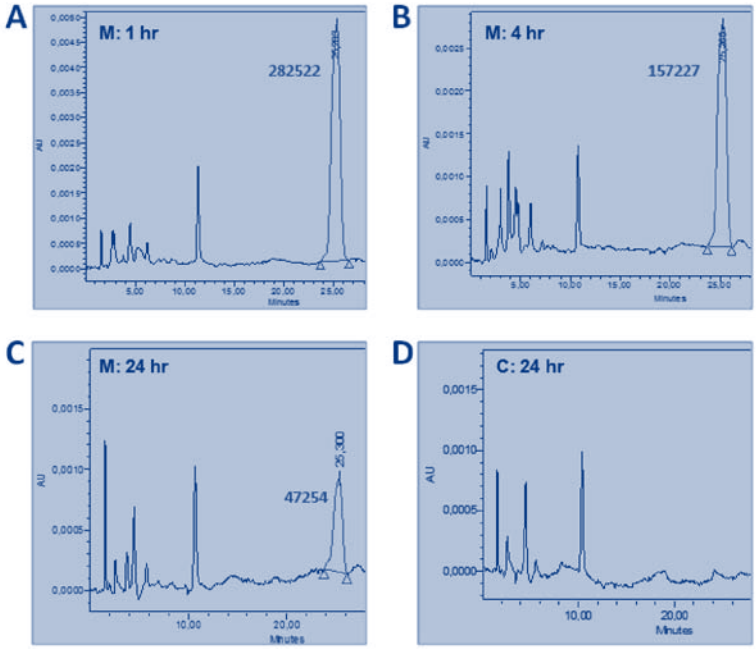


Figure 8 - HPLC analysis of medium before (A) or after incubation of 1 µM cortisol for 1 (B), 4 (C) or 24 (D) h with HepG2 cells. The results show the disappearance of cortisol (retention time 25 min) and generation of unidentified products, but no production of cortisone (retention time 23 min). Analysis of cell extracts also did not show production of cortisone (data not shown).

Discussion

The present study addressed the question of whether cortisol entry in liver cells takes place by simple diffusion or by means of transporter(s), using HepG2 cells as a model. Our results show that the uptake process is saturable, suggesting the presence of cortisol transporter(s). The uptake process is also temperature dependent but, unexpectedly, uptake was higher at 25°C than at 37°C. These findings do not allow a distinction to be made between diffusion and a carrier-mediated process, as both processes usually increase with temperature. The hypothetical cortisol transporter remains unidentified, as transfection of COS-1 cells with the candidate transporter OATP2B1 did not increase cortisol uptake.

It cannot be excluded that the observed cortisol uptake by HepG2 cells is determined to some extent by binding of cortisol to the cell surface or intracellular sites. This could also explain the higher cortisol uptake at 25°C than at 37°C. However it is also possible that both cortisol uptake and efflux are positively influenced by temperature, with a greater effect on efflux than on uptake. This may be envisaged if cortisol efflux is mediated by ABC transporter(s), whose activities are temperature dependent. This possibility is supported by the observation that not the initial cortisol uptake but rather its uptake after prolonged incubation (> 2 min) is higher at 37°C than at 25°C. Furthermore, cortisol uptake is enhanced by the efflux transporter inhibitor TC, suggesting that the net cellular uptake of cortisol is also determined largely by its efflux rate.

In the present study, medium pH, albumin and serum concentrations were varied to determine optimal conditions for cortisol uptake. The results tend to show maximum cortisol uptake in HepG2 cells at 0.1% BSA and pH 7.3. To test the possible effect of CBG on cortisol uptake, we added up to 10% serum to the incubation medium. However, this failed to affect cortisol uptake by HepG2 cells. This could be explained by the fact that under physiological conditions, 80-90% of serum cortisol is bound to CBG. Therefore, 10% FBS in the incubation medium may be insufficient to bind a significant fraction of cortisol.

As mentioned above, the effects of efflux inhibitors have also been tested on cortisol uptake by HepG2 cells. Only TC, which specifically blocks P-gp, shows an increase in cortisol uptake at 200 μM. Ver, which blocks both P-gp and BCRP, did not produce a significant increase in cortisol uptake. Therefore, the role of P-gp in cortisol efflux from HepG2 cells remains unclear.

Interestingly, BSP was found to inhibit cortisol uptake. BSP is not only a ligand for various efflux transporters, but also for multiple organic anion uptake transporters, in particular various members of the OATP family. Our results could therefore point to the involvement of organic anion transporters in hepatic uptake of cortisol, although we ruled out an important role for OATP2B1 in this respect.

It would be interesting to study the effects of synthetic glucocorticoids, such as prednisone and dexamethasone, on cortisol uptake by HepG2 cells. Due to their very similar molecular structure to cortisol, inhibition of uptake would only occur if cortisol is taken up by a carrier-mediated transport process.

There are several limitations to our study. For instance, we only tested the possible transport of cortisol by the OATP2B1 and not by alternative transporters. The hypothesis that OATP2B1 is involved in cortisol transport was based on the findings of the interaction of different steroids with this transporter [17]. However, other interesting transporters remain to be investigated. The inhibition of cortisol uptake in HepG2 cells by BSP suggests the involvement of other organic anion transporters such as OATP1B3. The latter is expressed exclusively in liver, and evidence suggests that it transports testosterone, another steroid hormone [18].

In conclusion, our study provides evidence that uptake of cortisol in HepG2 cells is saturable, suggesting that this process is mediated by transporter(s). Inhibition of cortisol uptake by BSP suggests the involvement of organic anion transporter(s), although OATP2B1 did not facilitate cortisol transport. Specific transporters capable of transporting cortisol remain to be identified. Various tissues may express different transporters with different specificities for cortisol analogs. This would allow the tissue-specific targeting of synthetic glucocorticoids with interesting pharmacological properties.

References

1. *Revollo JR, Cidlowski JA. Mechanisms generating diversity in glucocorticoid receptor signaling. Ann N Y Acad Sci. 2009; 1179:167-178*
2. *Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids--new mechanisms for old drugs. N Engl J Med. 2005; 353:1711-1723*
3. *Lewis JG, Bagley CJ, Elder PA et al. Plasma free cortisol fraction reflects levels of functioning corticosteroid-binding globulin. Clin Chim Acta. 2005; 359:189-194*
4. *Henley DE, Lightman SL New insights into corticosteroid-binding globulin and glucocorticoid delivery. Neuroscience 2011;180:1-8*
5. *Mendel CM, Kuhn RW, Weisiger RA et al. Uptake of cortisol by the perfused rat liver: validity of the free hormone hypothesis applied to cortisol. Endocrinology. 1989; 124:468-476*
6. *Seckl JR. 11beta-Hydroxysteroid dehydrogenase in the brain: a novel regulator of glucocorticoid action? Front Neuroendocrinol. 1997; 18:49-99*
7. *Funder JW. Glucocorticoid and mineralocorticoid receptors: biology and clinical relevance. Annu Rev Med. 1997; 48:231-240*
8. *Rao GS. MOde of entry of steroid and thyroid hormones into cells. Mol Cell Endocrinol. 1981; 21:97-108*
9. *Spindler KD, Kanuma K, Grossmann D. Uptake of corticosterone into isolated rat liver cells: possible involvement of Na+/K(+)-ATPase. J Steroid Biochem Mol Biol. 1991; 38:721-725*
10. *Jonkers GH, Timmermans HA, De Jong FH et al. An energy-dependent corticosterone uptake system in the rat liver cell. FEBS Lett. 1980; 111:403-406*
11. *Rao GS, Schulze-Hagen K, Rao ML et al. Kinetics of steroid transport through cell membranes: comparison of the uptake of cortisol by isolated rat liver cells with binding of cortisol to rat liver cytosol. J Steroid Biochem. 1976; 7:1123-1129*
12. *Hennemann G, Docter R, Friesema EC et al. Plasma membrane transport of thyroid hormones and its role in thyroid hormone metabolism and bioavailability. Endocr Rev. 2001; 22:451-476*
13. *Heuer H, Visser TJ. Minireview: Pathophysiological importance of thyroid hormone transporters. Endocrinology. 2009; 150:1078-1083*
14. *Gasser PJ, Lowry CA, Orchinik M. Corticosterone-sensitive monoamine transport in the rat dorsomedial hypothalamus: potential role for organic cation transporter 3 in stress-induced modulation of monoaminergic neurotransmission. J Neurosci. 2006; 26:8758-8766*
15. *Mason BL, Pariante CM, Jamel S et al. Central nervous system (CNS) delivery of glucocorticoids is fine-tuned by saturable transporters at the blood-CNS barriers and nonbarrier regions. Endocrinology 2010; 151:5294-5305*
16. *Matsson P, Pedersen JM, Norinder U et al. Identification of novel specific and general inhibitors of the three major human ATP-binding cassette transporters P-gp, BCRP and MRP2 among registered drugs. Pharm Res. 2009; 26:1816-1831*
17. *Grube M, Köck K, Karner S et al. Modification of OATP2B1-mediated transport by steroid hormones. Mol Pharmacol. 2006;70:1735-1741*
18. *Hamada A, Sissung T, Price DK et al. Effect of SLCO1B3 haplotype on testosterone transport and clinical outcome in caucasian patients with androgen-independent prostatic cancer. Clin Cancer Res. 2008; 14:3312-3318.*

Liquid based vs. conventional cytology for evaluation of fine needle aspiration biopsies obtained by pulmonary physicians *A pilot study*

Marlene Mende[a], Annemarie van Leeuwen[a], Marleen Bakker[b], Coenraad Koegelenberg[c]
[a] Medical student, Erasmus MC University Medical Center Rotterdam, the Netherlands
[b] Supervisor, Department of Pulmonology, Erasmus MC University Medical Center Rotterdam, the Netherlands
[c] Supervisor, Department of Pulmonology, University of Stellenbosch Tygerberg Academic Hospital, South Africa
Correspondence: Marlene Mende, e-mail: 303959mm@student.eur.nl

Background: **Transthoracic fine needle aspiration (TTFNA) of pulmonary and mediastinal pathology is a well-established modality that is often augmented by the use of rapid on-site evaluation (ROSE). Liquid based cytology (LBC), which has been validated for cervical cancer screening, has many potential advantages as a rapid evaluation method.**
Aim: **Because the use of LBC has never been validated for evaluation of TTFNA biopsies, we aimed to compare the diagnostic yield of LBC compared to conventional cytology in the evaluation of intrathoracic mass lesions.**
Methods: **TTFNA was performed on 30 consecutive patients (59.0 SD 11.1 years, 20 males). Three passes were presented for rapid on-site evaluation and a fourth pass for LBC. A cutting needle biopsy (CNB) was performed if an on-site diagnosis of epithelial carcinoma or tuberculosis (TB) could not be established.**
Results: **Final diagnoses included epithelial carcinomas (n=26), other malignancies (n=3) and TB (n=1). Conventional cytology on-site yielded a diagnosis in 26 patients (86,7%), whereas LBC was diagnostic in 11 patients (36.7%, p<0.001).**
Conclusions: **TTFNA with LBC has a diagnostic yield inferior to conventional cytology. Due to limitations in our study, further research is needed to accurately define the value of LBC in the evaluation of intrathoracic masses.**

Introduction

Transthoracic fine needle aspiration (TTFNA) of pulmonary and mediastinal specimens is a well-established modality that is often augmented with rapid on-site evaluation (ROSE) [1-5]. Numerous studies have found the diagnostic yield of TTFNA with ROSE to be in excess of 90% for epithelial carcinoma of the lung, which is comparable to more invasive procedures [1-5]. Furthermore, TTFNA has a low complication rate, with pneumothoraces observed in only 1% of cases [3].

Liquid based cytology (LBC) is an exciting new technology developed for cervical cancer screening [6]. LBC has been used almost exclusively for the assessment of gynecological specimens, where it has been shown to reduce the number of false-negative results [6]. It does this by enhancing the quality of the conventional smear through an improved slide preparation technique following collection of the same proportion of the sample in the standard way. This produces a more representative sample of the specimen, with less obscuring background material. In principle, this should allow faster and more reliable screening by laboratory staff. Moreover, the preservation fluid used in LBC allows TTFNA biopsies to be evaluated rural areas where rapid on-site evaluation is not available. The evaluation of the LBC can then be performed at a later time and different location.

LBC does not involve making a smear of the material obtained. Instead, the material is rinsed in a specifically prepared preservative fluid, thus generating a suspension of cells that is subsequently used to deposit a monolayer of cells on the slide. The preservative fluid that is used in LBC for non-gynecological specimens is called BD CytoRich™ non-gyn Red Preservative. Almost all of the cells collected should therefore be present in the fluid. After processing, a smaller but more representative cell sample is obtained than with a conventional smear. Cellular preservation is thought to be enhanced, the preparation is closer to an actual monolayer and contamination (blood cells, pus and mucus) is reduced [6]. Moreover, improved fixation allows more consistent staining. As a result, it is claimed that this preparation technique reduces the proportion of specimens classified as technically unsatisfactory for evaluation. A further advantage is that the cell suspension can be retained and used for later testing, such as immunohistochemistry and other molecular biological tests. Finally, these new preparation techniques could greatly facilitate the introduction of automated analytical methods [6].

The diagnostic yield of BD CytoRich™ non-gyn preparation of specimens obtained by means of fine needle aspiration is currently unknown. Our aim was therefore to compare the diagnostic yield of this method with conventional preparation techniques when used on intrathoracic mass lesions.

Methods

Study Population

Consecutive patients (≥18 years of age) with an intrathoracic mass lesion abutting the chest wall and a high clinical and radiological suspicion of lung cancer were invited to participate in this pilot study. Inclusion criteria were that an interface of at least 1cm in two dimensions was present, ultrasound assisted TTFNA was deemed to be the investigation of choice by the attending clinician and no known coagulopathy was present.

Our study took place at Tygerberg Academic Hospital, a 1,200-bed university hospital in Cape Town, South Africa. It is one of two referral centers and provides tertiary service to a population of approximately 1.5 million people. The Health Research Ethics Committee of Stellenbosch University approved the study. Written informed consent was obtained from all subjects on enrolment and also prior to any invasive procedures.

Transthoracic ultrasound
A consultant respiratory physician performed the ultrasonography (Toshiba Just Vision 200 SSA-320A; Toshiba Medical Systems Corporation, Tochigi-ken, Japan) in a bronchoscopy suite. The interface between the intrathoracic mass lesion and chest wall was identified. An interface of at least 1cm in two dimensions visible throughout inspiration and expiration in an area not covered by bony elements was considered the minimum requirement for an ultrasound-assisted procedure.

Transthoracic fine needle aspirations
Aspirations were performed with 22-G spinal needles, 40mm or 90mm in length as needed (Tae-Cang, Kong Ju City, Korea), connected to a 10 ml syringe under sterile conditions with local anesthesia (lignocaine 1%). Aspirates from at least three slightly different directions and depths were obtained and immediately expressed onto numbered glass slides. After this a fourth pass was taken and washed into a specifically prepared vial containing BD CytoRich™ (Franklin Lakes, NJ, USA). The rapid modified Wright–Giemsa stains were performed on unfixed air-dried slides. This procedure involved three steps and took 45 seconds to complete. The rapid Papanicolaou stains were spray-fixed (ether/alcohol with poly-ethylenei-glycol; Fencott, Sangene Products, Cape Town, South Africa) and stained on-site with a procedure involving 15 steps, which took 3 minutes to complete.

Rapid on-site evaluation (ROSE) of cytology specimens
The cytopathologist present in the operating theater was asked to comment on the presence of diagnostically useful material obtained and to provisionally type the diagnostically useful specimens as one of four main categories: (1) epithelial carcinomas of known origin, (2) other malignancies, (3) probable tuberculous disease (necrotizing and/or granulomatous inflammation) and (4) other benign pathology. We considered specimens with a provisional on-site diagnosis of either epithelial carcinomas of known origin or probable tuberculosis as sufficient for a possible final cytological diagnosis (i.e. no histology was obtained). In case of malignancies other than epithelial carcinoma, the cytopathologist was asked to make a provisional diagnosis, if possible, and to suggest further tests, for example flow cytometry for non-Hodgkin's lymphoma or aspirates for mycobacterial cultures in case of suspected tuberculosis.

Cutting needle biopsies
Cutting needle biopsies (CNB) were obtained during the same session immediately following TTFNA in all cases where the provisional on-site diagnosis was not an epithelial carcinoma of a known origin or tuberculosis, provided that a safety range of at least 1 cm could be assured (i.e. no mediastinal organs or blood vessels within 1cm of the intended CNB path). Manually operated 14-gauge Trucut biopsy needles (Allegiance, Chateaubriand, France) were used. Two or more passes were performed until macroscopically satisfactory material was harvested. These specimens were harvested in 4% formalin and routinely processed for histological evaluation.

Immediate post-procedure care
The TTFNA and CNB site were re-examined by means of ultrasound immediately after the procedures, and a chest radiograph was obtained at the discretion of the attending physician if the pre- and post-procedure ultrasound findings differed. All patients were observed for at least two hours prior to discharge. Minor or major hemorrhages, as well as iatrogenic pneumothoraces were documented. Major hemorrhage was defined as any hemorrhage that required additional measures other than localized pressure and superficial sutures.

Further assessment of conventional cytology
All cytology slides were reviewed in the laboratory by an independent cytopathologist who had access to an array of special stains (including immunohistochemistry). A final cytological diagnosis was issued after concurrence was reached.

Processing of specimens via the BD Diagnostic systems
Specimens were homogenized by vortexing (Fisons Tube Vortex Whirlimixer) for 10 seconds. After removal of clots and particles, specimens were centrifuged at 1,500 rpm for 10 minutes. The supernatant was discarded and 10 ml BD Cytorich was added (Cytorich Red Preservative, Tripath Imaging INC. Burlington. NC. USA). Fixation was obtained by vortexing. The specimens were then transferred to the tubes suitable for the Rotina 380 Centrifuge. (Hettich Centrifuge ZEN TRIFUGEN), centrifuged for 10 minutes at 600 rpm and decanted. After 10 ml di-ionized water was added, they were centrifuged for 5 minutes at 600 rpm and decanted.

The labeled tubes were loaded in the BD Prepstain with the corresponding BD Surepath precoated slides. The slides were prepared according to the BD Prepstain operator's manual after checking the Prepstain 0.5 hematoxilin stain and the ae/orange-G combo stain levels (Tripath Imaging INC. Burlington NC USA). The slide racks were removed from the Prepstain, and the slides were rinsed with Prepstain alcohol blend rinse (Tripath Imaging INC. Burlington, NC, USA). The BD CytoRich™ non-gyn preparation was completed by 100% alcohol dipping, clearing with Zylene and cover slipping.

Three cytopathologists, blinded to the on-site findings and the identity of the patients, reviewed the samples, and had to concur prior to issuing cytological diagnosis.

Histology
The histological specimens were reviewed by two independent pathologists. Histology was classified as "diagnostic" or "non-diagnostic" (normal tissue, not representative tissue, or representative but necrotic tissue). Only histological diagnoses or unequivocal cytology were accepted as diagnostic and used as the gold standard for statistical analyses.

If the initial procedures failed to yield diagnoses, these patients underwent further tests. The choice for additional invasive procedures was guided by the patients' attending chest physician, and could include a second bronchoscopy, CT-guided biopsies, mediastinoscopy or open surgical procedures.

Statistical analysis
Descriptive statistical analyses were performed, and McNemar's test was used to compare the diagnostic sensitivities of conventional preparation techniques and LBC. Unless stated otherwise, data are displayed as means ± standard deviation (SD).

Results

Patients and final diagnoses
Over a three-month period (October – December 2011) we enrolled 30 patients (59.0 +/- 11.1 years, 20 males). No patients were excluded or declined to give consent. In total, 24 patients were

diagnosed with bronchogenic carcinoma (Table 1), with adenocarcinoma (n=10) and squamous cell carcinoma (n=8) being the most common diagnoses.

Transthoracic needle aspiration
TTFNA was performed on all enrolled patients, and diagnostically useful material was confirmed by the on-site pathologist in 26 cases (86.7%). The laboratory diagnosis concurred with the final diagnosis in all 26 cases, including all cases of neoplastic disease other than lung cancer (all diagnosed on histological specimens). The only cases that escaped diagnosis were 2 cases of squamous cell carcinoma, a single case with spindle cells (uncertain final diagnosis) and a single case of pulmonary TB.

Liquid-based cytology
LBC was diagnostic in 11 cases (36.7%), significantly less than conventional cytology (p<0.001). LBC was diagnostic only in cases of epithelial carcinomas and missed all other diagnoses. One case of squamous cell carcinoma (confirmed by histology) was diagnosed with LBC alone (not with conventional cytology), and one case of small cell lung cancer (confirmed with immunocytochemistry) was erroneously reported as an adenocarcinoma. Ten cases contained atypical cells, but too few cells were present to make an accurate diagnosis.

Trucut biopsies
Trucut biopsies were obtained in all cases where the on-site findings were not compatible with epithelial carcinoma (n=4) or TB (n=0), or where no diagnostic material was present (n=2). Histology from these biopsies confirmed the diagnoses in 4 cases (66.7%). The single case of pulmonary TB yielded non-diagnostic material, and the histology on another patient yielded only a spindle cell tumor that could not be typed.

Complications and additional tests
One patient experienced self-limiting mild hemoptysis following the final TTFNA pass (no intervention required). One patient required a bronchoscopy to confirm the final diagnosis (pulmonary tuberculosis), and one patient died of a stage 4 spindle cell tumor that was never accurately typed.

Discussion

To the best of our knowledge, this pilot study is the first to investigate the use of LBC in the evaluation of intrathoracic masses sampled by TTFNA. We found that LBC of TTFNA specimens has a disappointingly low diagnostic yield, inferior to that of conventional cytology (36.7% vs. 86.7%, p<0.001). Although we included a relatively small number of patients, we conclude that this finding is highly significant. Including more patients would not alter the results in face of such strong evidence of a difference in outcome (p<0.001) [8,9].

Table 1 - Final established diagnoses of all study subjects (n=30)

Diagnosis	n	%
Non-small cell lung cancer		
Adenocarcinoma	10	33.3
Squamous cell carcinoma	8	26.7
Undifferentiated/large cell	5	16.7
Small cell lung cancer	1	3.3
Metastatic adenocarcinoma (not lung)	2	6.7
Non Hodgkin's Lymphoma (large B-cell)	1	3.3
Plasmacytoma	1	3.3
Spindle cell tumor	1	3.3
Pulmonary tuberculosis	1	3.3

Nevertheless, these results could have been affected by a major limitation of our study: the total volume of material aspirated for the LBC method was not equal to that aspirated for routine cytology. This undoubtedly disadvantaged LBC. Because we were using a method (LBC) with unknown diagnostic value, we minimized discomfort and risk for patients by performing only a single pass for LBC. This may have biased the comparison of the diagnostic yields, as the single pass for LBC was compared with three passes of conventional cytology (augmented by ROSE). If we had used a larger volume of material, it is likely that LBC would have been diagnostic in more cases. For example, cellular atypia were present in at least 10 cases, but these were reported as containing "too few cells to make a reliable cytological diagnosis," which is probably a direct consequence of the smaller volume. If the additional 10 cases had been positive, the diagnostic yield could have been as high as 21/30 (70.0%); in our study this would not have been significantly different than the conventional method.

In conclusion, the general use of LBC for specimens obtained is clearly not advisable at present, but it should not be discarded either based solely on our data. We therefore suggest additional randomized studies that use equal volumes of aspirated material for both LBC and conventional cytology.

ACKNOWLEDGMENTS
We would like to thank the staff of the Division of Pulmonology, Department of Medicine and the staff of the Division of Anatomical Pathology, Department of Pathology, of the Tygerberg Academic Hospital for their excellent collaboration.
CONFLICT OF INTEREST
Nothing to declare.
FUNDING
The Division of Anatomical Pathology, Department of Pathology received funding from the K-funding (internal research fund) specifically for the preparation of the LBC specimens, as this was not routinely done in the laboratory at the time of the study.

APPENDIX
1. List of abbreviations

LBC	Liquid based cytology
TTFNA	Transthoracic fine needle aspiration
ROSE	Rapid on-site evaluation
CNB	Cutting needle biopsy
TB	Tuberculosis
SD	Standard deviation

References

1. *Koegelenberg CF, Bolliger CT, Plekker DP, et al. 2009. Diagnostic yield and safety of ultrasound-assisted biopsies in superior vena cava syndrome. Eur Respir J 33:1389-1395.*
2. *Schubert P, Wright AC, Louw M, et al. 2005. Ultrasound-Assisted Transthoracic Biopsy: Cells or Section? Diagn Cytopathol 33:233-237.*
3. *Diacon AH, Theron J, Schubert P, et al. 2007. Ultrasound-assisted transthoracic biopsy: fine-needle aspiration or cutting-needle biopsy? Eur Respir J 29:357-362.*
4. *Koegelenberg CFN, Bolliger CT, Irusen EM, et al. 2011. The diagnostic yield and safety of ultrasound-assisted transthoracic fine needle aspiration of drowned lung. Respiration 81:26–31.*
5. *Koegelenberg CF, Bolliger CT, Irusen EM, et al. 2011. The diagnostic yield and safety of ultrasound-assisted biopsy of mediastinal masses. Respiration 81:134-141.*
6. *Karnon J, Peters J, Platt J, et al. 2004. Liquid-based cytology in cervical screening: an updated rapid and systematic review and economic analysis. Health Technology Assessment; 8:1-78*
7. *Keebler KM: Cytopathology techniques; In Bibbo M (ed): Comprehensive Cytopathology. 2nd Edn. Philadelphia, WB Saunders, 1997; pp. 881-906*
8. *Haybittle J. Repeated assessment of results in clinical trials of cancer treatment. Br J Radiol 1971;44:793-7.*
9. *Peto R, Pike M, Armitage P. Design and analysis of randomized clinical trials requiring prolonged observation of each patient: introduction. Br J Cancer 1976;34:585-612.*

What is the role of the cerebellum in language?
A systematic review

Subramanian Venkatesan
Medical student, Erasmus MC University Medical Center Rotterdam, the Netherlands
Correspondence: Subramanian Venkatesan, e-mail: 340980sv@student.eur.nl

Objective: **To assess the presence of a cerebellar component in language and to investigate a lateralization of language function in the cerebellum and the specific quality of this function.**
Methods: **I systematically reviewed the literature on the role of the cerebellum in language. I only included controlled studies with patients suffering from isolated cerebellar damage and studies which used one or more neuropsychological tests to evaluate the influence of the cerebellum on linguistic functions.**
Results: **Four articles met the inclusion criteria. Two of these articles did not show evidence for a cerebellar language function. One study showed a marked deficit only in the right cerebellar damaged group compared to the control group in all neuropsychological tests related to verbal function. However, another study showed a substantial deficit in speech production only for the left-hemisphere damaged group and in the grammaticality judgment task in both the left- and right-hemisphere damaged groups.**
Conclusions: **The cerebellum probably contains a certain language function, but this appears to be subtle. Further studies are needed to clarify the possible lateralization of language function.**

Introduction
A general misconception about the cerebellum is that it is solely responsible for motor coordination. However, research has provided evidence of possible higher cognitive functions in the cerebellum[1]. In particular, the theory "cerebellar cognitive affective syndrome"[2] has drawn a lot of attention. Nevertheless the precise role of the cerebellum in language function is still unclear. Clarifying this role could have clinical implications such as surgery involving the cerebellum or for diagnosing language deficits.

Several major theories on the cerebellar contribution to language can be found in the literature. One of these theories, "Dysmetria of thought hypothesis", also known as 'the timing hypothesis", states that the cerebellum does not directly influence language, but plays a role in the timing of linguistic functions (3). Cerebellar damage could impair temporal modulation, which is necessary for the application of syntactic rules and other functions, therefore leading to a less controlled language function. According to another theory, "cerebellocerebral diaschisis", a cerebellar lesion affects a distant, but anatomically connected cerebral region(4); the cerebellum has a modulatory role in a multi-component neural circuit. Tracer studies have shown that a vast number of connections exist between a cerebellar hemisphere and its contralateral cerebral hemisphere. Because the right cerebral hemisphere contains these functions, this implies that left cerebellar lesions would result in motor and visuospatial deficits and that right cerebellar lesions would result in prominent language deficits.

A third theory, the "ataxic dysarthria theory" posits that the cerebellum mainly controls the motor aspects of language production. Therefore a cerebellar lesion would lead primarily to slurred speech.

In this review the following research questions were addressed: is a language component present in the cerebellum and is there a lateralization of this function in a cerebellar hemisphere?

Methods
The inclusion and exclusion criteria for this review concerned 1) types of studies, 2) types of participants, 3) types of assessment and 4) primary and secondary outcome measures.

Types of studies
Only comparative studies investigating cerebellar language function written in English regardless of the date of publication were included.

Types of participants
Studies with subjects with an acquired cerebellar focal lesion were included. Studies involving patients with pre-existent or concurrent neurologic disorders or deficits other than the cerebellar lesions were excluded, as were studies investigating subjects who had extra-cerebellar lesions or who received cranial radiotherapy. The controls had to be healthy (no neurological impairments). No restrictions were made based on age or sex. Only studies were included which provided information on the location of the cerebellar lesion.

Types of assessment
The studies were required to contain information on the left- and right-handedness of the subjects and controls. Furthermore the studies had to include one or more neuropsychological tests to evaluate the possible influence of the cerebellum on language. The neuropsychological tests had to be specifically designed tasks that measure the psychological function known to be linked to language. No restrictions were made on what kind of language component was examined.

Types of outcome measures
Primary outcomes:
The results of the neuropsychological tests assessing the presence of a language function in the cerebellum in general were the primary outcomes.

Secondary outcomes:
The results of any neuropsychological test in relation to the possible lateralization of a language function in the left or right cerebellar hemisphere were the secondary outcomes.

Search methods for appropriate studies
Electronic Search
The following search terms were used in PubMed on the 15th of January 2012:
("cerebellum "[MAJR] OR "cerebellar diseases"[MAJR]) AND ("speech"[MeSH] OR "language disorders"[MeSH] OR "verbal behavior"[MeSH]) AND "psychological tests"[MeSH] AND "humans"[MeSH] AND (Clinical Trial[ptyp] OR "epidemiologic studies"[MeSH]) NOT "review"[publication type] AND (lopro-vinleurlib OR free full text[3])
No further limits were used.

Data collection and analysis
Selection of studies
During the first selection round the clinical trials using the described inclusion and exclusion criteria (types of studies, participants, assessment and outcome measures) were selected based on the title and the abstract. During the second selection round studies were excluded based on the full text using the same criteria.

Data extraction and synthesis
The following information from each study was collected and placed in a table:
1. description of the study, including the first author, the year of publication, the sample size;
2. participant characteristics, including the location of the cerebellar lesion and a further specification of the patients' condition at assessment;
3. the applied neuropsychological tests, including the p-value of the group comparisons between the cerebellar patients and the control group as well as the explanatory theory.

Risk of bias assessment
The presence of possible confounders were investigated, including confounding characteristics of the subjects which could distort the possible relationship between cerebellar lesions and language.

Results

Description of included studies
The PubMed search term yielded 26 publications (Figure 1). After the first selection round, 12 potentially relevant articles remained. After the second round, 4 articles were included in the qualitative analysis of this systematic review (Table 1).
The 4 studies included a total of 70 subjects with 86 controls matched for at least age and education. In total, 27 subjects had left cerebellar damage (LCD), 30 subjects had right cerebellar damage (RCD),11 subject had bilateral cerebellar damage (BCD) and 2 had medial cerebellar damage (MCD). However, the data from Justus et al. on 9 subjects with BCD were excluded from this analysis, since they had a degenerative or genetic disorder.
The studies of Frank et al. and Richter et al. originated from the same research group. In Richter et al., all the patients had chronic cerebellar lesions, while in Frank et al. the subjects all presented with a unilateral stroke less than 8 weeks earlier. The same research group therefore studied patients with chronic cerebellar diseases in 2005 and acute cerebellar diseases in 2010. This explains the similarity of the neuropsychological tests that were applied.

Risk of bias assessment
Possible confounders
Using preoperative MRI, Gottwald et al. observed 9 cases with slight brainstem compression and 7 cases with a slight hydrocephalus. Although a slight brainstem compression can be recognized as an extra-cerebellar, I included the data on these patients in the analysis. This is because hydrocephalus [4, 5] and brainstem compressions [6] are subtle defects which may have been unrecognized in the other studies.

Measure of cerebellar influence
Primary outcomes
Both Richter et al. and Frank et al. did not report a significant difference in any of their applied neuropsychological tests between cerebellar patient groups and their matched controls. Both studies use the following tests: verb generation, aachener aphasia test, speech motor task, qualitative speech analysis. Richter et al. also used the sprachentwicklungstest.
Patients in the studies that included both acute and chronic cerebellar lesions performed within the normal range of the tests. Gottwald et al. used the following tests: wechsler memory scale revised (a verbal memory subtest), verbal fluency (testing semantic and phonematic fluency), stroop test (testing color word reading and color naming interference). Gottwald et al. reported a marked deficit compared to the control group only in the right cerebellar damaged group (RCD) in all neuropsychological tests related to verbal function. Justus et al. used the following tests: speech production, language comprehension task, grammaticality judgment task. Justus et al. reported impairment in grammatical morphology across speech production and grammaticality judgment tasks (Table 2).

Figure 1 -
Flow of information through the search phases

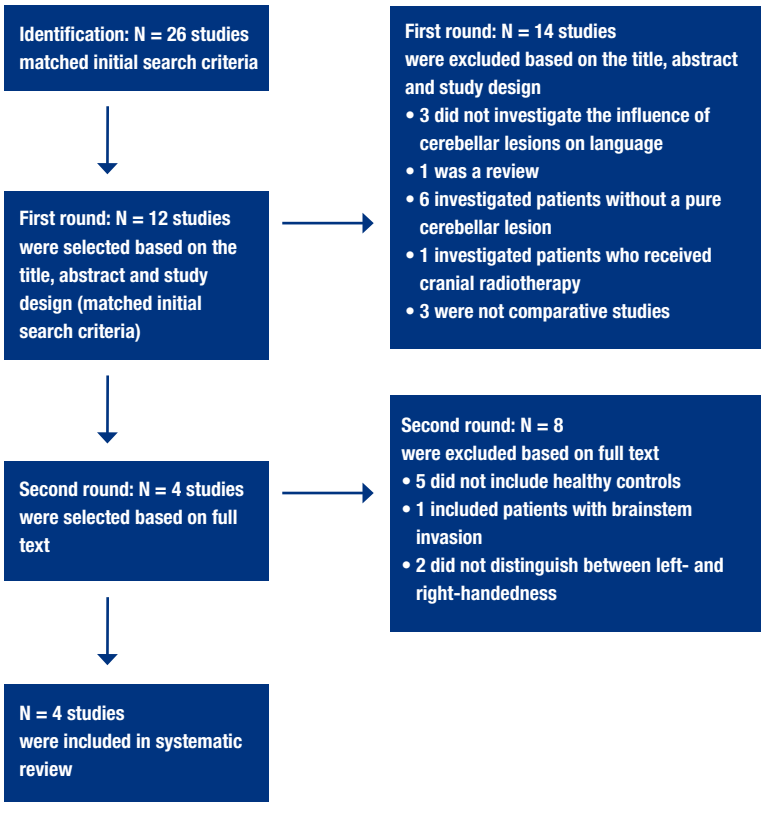


Table 1 - Characteristics of included studies

Study	Sample size Subject/ control	Cerebellar condition LCD	BCD	RCD	MCD	Subjects further specified	Neurolinguistic test	Group comparison	Supporting theory
Frank et al (2010)(1)	22/22	12	-	10	-	- All presented with lesions including parts of the posterolateral hemispheres (i.e. lobules VI or below)- All patients had a unilateral stroke less than 8 weeks ago	Verb generation Aachener aphasia test Speech motor task Qualitative speech analysis	NS NS NS NS	- Against the presence of a language function
Richter et al (2005)(2)	12/27	4	1	6	-	- All patients underwent a cerebellar operation to remove an astrocytoma at least more than one year ago	Verb generation Aachener aphasia test Speech motor task Qualitative speech analysis Sprachentwicklungs-test	NS NS NS NS NS	- Against the presence of a language function
Gottwald et al (2004)(3)	21/21	8	1	11	1	- Preoperative MRI showed in nine cases a slight brainstem compression and in seven cases a slight hydrocephalus. - 14 were assessed pre-operatively and 7 post-operatively	Wechsler Memory Scale Revised: Verbal memory subtest Verbal fluency: Semantic fluency Phonematic fluency Stroop-test: Color word reading Color naming Interference	 P=0,030* P=0,000* P=0,002* P=0,002* P=0,002* P=0,036*	- Supporting the cerebellocerebral diaschisis theory - dysmetria of thought
Justus (2004)(4)	16/16	3	9	3	1	- The data on the 9 patients with BCD were not included in this meta-analysis, since they had degenerative or genetic disorders. - 4 had a tumour and 3 had a unilateral stroke	Speech production Language comprehension task Grammaticality judgment task	P=0,046*** NS P<0,001** P<0,001*	-against a lateralization of a specific language function -speech perception function

NS= no significant difference between the cerebellar patient groups (LCD, BCD, RCD or MCD) and the matched control groups
*Group comparison: overall performance on the task, the controls performed better than the RCD. The other cerebellar patient groups:NS, unless stated differently
** Group comparison: overall performance on the task, the controls performed better than the LCD. The other cerebellar patient groups:NS, unless stated differently
*** Group comparison: the controls produced significantly more required articles than the LCD. The other cerebellar patient groups: NS
LCD left cerebellar damage, BCD bilateral cerebellar damage, RCD right cerebellar damage, MCD medial cerebellar damage

Secondary outcomes

Richter et al. and Frank et al. showed no presence of language function in the cerebellum.

Gottwald et al. reported that 6 out of the 6 subtests assessing language showed a significant deficit for the patients with the RCD compared to their matched controls. The same tests did not show any deficits in the LCD group compared to the control group. The RCD group performed significantly worse on the verbal memory subtest of the wechsler memory scale-revised, verbal fluency and the stroop test. Justus et al. reported a significant deficit in speech production only for the LCD patients and in the grammaticality judgment task in both the LCD and RCD patients.

Discussion

Current literature cautiously attributes a cerebellar contribution to language. The lateralization of a possible language function and the precise role of this contribution are unclear.

Cerebellar language function

Of the 4 reviewed studies, 2 showed cerebellar language function [7, 8] and 2 did not [9, 10]. The last 2 studies [9, 10] were conducted by the same research group using largely the same tests. A possible explanation for the discrepancy in results is that these studies [9, 10] did not use sufficiently sensitive tests to evaluate language deficits. For example, these studies used the verb generation test to assess the executive function in relation to language, while Gottwald et al. used a verbal fluency test. Furthermore, the sprachentwicklungstest used in Richter et al. proved to be very aspecific in detecting grammatical dysfunction since the controls themselves scored below normal test range. In contrast the grammatical morphology tests used in Justus et al. are more sensitive and specific (i.e. the patients scored low and the controls scored consistently high). Apart from the sprachentwicklungstest, the tests used in Richter et al. and Frank et al. seem well-constructed (i.e. the controls scored consistently high), but did not seem sensitive enough to detect language deficits since their tests did not find significant changes whereas the tests of Justus et al. and Gottwald et al. did.

Table 2 – explanation of the applied neurolinguistic tests

Neurolinguistic test	Applied in study	explanation
Wechsler Memory	[8]	- Measures the attentional capacity or very short term memory in relation to
Scale Revised (WMS-R): verbal memory subtest		spoken words
Verbal fluency: semantic and phonematic verbal fluency	[8]	- A 'free-recall test'
		- Arguably more challenging and more sensitive that the verb generation test
Stroop test	[8]	- Examines the fluency of color word reading and the effect of color naming
		Interference
		- Examines the executive functions related to language.
Speech production: picture description task	[7]	- Tests the use of grammatical morphemes and canonical word order in
		response to describing a series of pictures depicting simple events
Language comprehension task	[7]	- The task is to listen to an aurally presented sentence and to pick out the
		agent in a sentence(i.e. what performed the action?)
Grammaticality judgment task	[7]	- The task is to listen to an aurally presented sentence and determine
		whether the sentence was grammatically correct or incorrect
Verb generation: naming condition, verb-generation condition	[9-10]	- Examines the executive functions related to language
		- Arguably less sensitive than the verbal fluency test
Aachener aphasia test: token test and written language	[9-10]	- Examines the executive functions related to language.
subtest		
Heidelberger Sprachentwicklungstest (HSET): sentence	[9-10]	- A sentence has to be constructed from two or three words
construction subtest and plural-singular formation subtest		- To generate the plural form from the singular form or vice versa of words
Speech motor task: qualitative speech analysis: dysarthria	[9-10]	- Examines the measure of dysarthria (i.e. the lack of good articulation)
symptom scale		

These results are important, since they indicate that many tests were not able to detect a language deficit and that cerebellar damage likely does not lead to overt language deficits. On the contrary, subtle language deficits are more likely to develop due to cerebellar damage.

Lateralization of language function

Due to the heterogeneity of the results, it is difficult to determine the location in the cerebellum where the language function is lateralized. The studies by Frank et al. and Justus et al. [9, 10] suggested no significant language function, while Gottwald, et al. [8] suggested a right-sided language function, whereby the right-cerebellar hemisphere could have a function in language in the aspects of memory (explained by the verbal memory subtest) and executive functions (explained by the verbal fluency test and the stroop test). One study [7] suggested a bilateral language function based on a significant deficit in speech production that was found only for the LCD patients and in the grammaticality judgment task in both the LCD and RCD patients. This heterogeneity can probably be explained by the large variety of applied tests in the different studies. Furthermore, all the included studies had a limited number of subjects with a unilateral lesion. In total, only 27 left-hemisphere and 30 right- hemisphere patients were included.

Specific language function

When considering the specific cerebellar contribution to language function, Gottwald et al. suggested a cerebellar language function in the aspects of memory and executive functions. Frank et al. and Richter et al. also used tests which examine executive functions related to language (i.e. verb generation and the token test in the aachener aphasia test) but neither study found a significant impairment. I previously discussed the possible reason for this discrepancy. The impaired executive function supports the dysmetria of thought hypothesis.

The impaired verbal memory supports the idea that the cerebellum contributes to modulating language function via verbal working memory and therefore also supports the dysmetria of thought hypothesis. However the results of Gottwald et al. should be interpreted cautiously, since nearly half of the right-hemisphere patients had a mild hydrocephalus and mild brainstem compression. Both hydrocephalus [4, 5] and brainstem compressions [6] can contribute to cognitive impairment, including language function. Gottwald et al. was the only included study to investigate verbal memory, so no decisive conclusions can be made about a cerebellar memory function in language.

Justus et al. reported that LCD patients have speech production deficits; when describing a picture, they omitted the use of required articles (i.e. "a", "an", "the") significantly more often than controls. This observation can be explained by the postulated ataxic dysarthria [11]. Because a problematic coordination of speech may lead to an efficient strategy in reducing speech, Justus et al. therefore appears to show a motor influence in speech only in LCD patients, and therefore supports the cerebellocerebral dischiasis theory. Furthermore this study suggests a role for the cerebellum in speech perception since both LCD and RCD patients showed a significant decrease in the ability to distinguish ungrammatical from grammatical sentences.

Conclusion

Based on the studies in this review, no definitive conclusions can be drawn about a language component or a lateralization of a language function in a cerebellar hemisphere. An important contributing factor to these inconclusive results is the lack of qualifying (i.e. without confounding characteristics) subjects. Perhaps more subtle language deficits can be elucidated with larger controlled studies with appropriate subjects. The included studies tend to show that the cerebellum does have a cerebellar language function. However, this contribution is probably subtle.

Future research should also pay more attention to the possible confounders for each included subject. For example, Gottwald et al. provided clear information on the fact that some patients presented with a hydrocephalus and/or a brainstem compression, while other studies did not mention anything about this. Finally, it should be stressed that all future studies should blind the observer, since bias can easily be introduced due to the large number of tests.

References

1. *Schmahmann, J.D., An emerging concept. The cerebellar contribution to higher function. Arch Neurol, 1991; 11: 1178-87.*
2. *Timmann, D. and I. Daum, Cerebellar contributions to cognitive functions: a progress report after two decades of research. Cerebellum, 2007; 3: 159-62.*
3. *Langer, T., et al., Floccular efferents in the rhesus macaque as revealed by autoradiography and horseradish peroxidase. J Comp Neurol, 1985; 1: 26-37.*
4. *Aarsen, F.K., et al., Long-term sequelae in children after cerebellar astrocytoma surgery. Neurology, 2004; 8: 1311-6.*
5. *Ronning, C., et al., Persistent cognitive dysfunction secondary to cerebellar injury in patients treated for posterior fossa tumors in childhood. Pediatr Neurosurg, 2005; 1: 15-21.*
6. *McMillan, H., et al., Brainstem compression: a predictor of postoperative cerebellar mutism. Child's Nervous System, 2009; 6: 677-681.*
7. *Justus, T., The cerebellum and English grammatical morphology: evidence from production, comprehension, and grammaticality judgments. J Cogn Neurosci, 2004; 7: 1115-30.*
8. *Gottwald, B., et al., Evidence for distinct cognitive deficits after focal cerebellar lesions. J Neurol Neurosurg Psychiatry, 2004; 11: 1524-31.*
9. *Frank, B., et al., Aphasia and neglect are uncommon in cerebellar disease: negative findings in a prospective study in acute cerebellar stroke. Cerebellum, 2010; 4: 556-66.*
10. *Richter, S., et al., Children and adolescents with chronic cerebellar lesions show no clinically relevant signs of aphasia or neglect. J Neurophysiol, 2005; 6: 4108-20.*
11. *Ackermann, H. and I. Hertrich, The contribution of the cerebellum to speech processing. Journal of Neurolinguistics, 2000; 2–3: 5-116.*

The association between non-adherence to immunosuppressive agents and graft function in renal transplant patients *A systematic review*

Marije Notenboom[a], Eva Schippers[a], Anne-Marlijn Versnel[a], Nienke Wolfhagen[a], Lilian Mohammad Zadeh[a], Emma Massey[b] and Marry de Klerk[b]
[a]Medical student, Erasmus MC University Medical Center Rotterdam, the Netherlands
[b]Supervisors, Department of internal medicine, Erasmus MC University Medical Center Rotterdam, the Netherlands
Correspondence: Marije Notenboom, email: 333092cn@eur.nl

Objective: **The aim of this study is to determine the effect of non-adherence to immunosuppressive agents on acute rejection, chronic rejection, graft failure, graft survival and allograft loss.**
Methods: **Pubmed database was searched from 2001 until January 2012 for original studies about non-adherence and graft loss in renal transplant patients. Articles were screened by two independent reviewers, starting with the abstract, taking into account the in- and exclusion criteria, followed by a full-text screening to determine the final set of articles. We analyzed this final set to determine its quality.**
Results: **The literature search identified 63 articles, of which 10 articles were included based on predetermined criteria. We found a significant correlation in 6 articles between non-adherence and acute rejection and in 2 articles between non-adherence and chronic rejection. A number of 3 articles showed significantly higher risk for graft failure and out 4 articles concerned allograft loss, 3 articles found a significant correlation. We also found weak evidence on the association between non-adherence and graft survival.**
Conclusion: **Non-adherence increases acute rejection, chronic rejection, graft failure and graft loss. Non-adherence might decrease graft survival. In this systematic review we have shown the importance of preventing non-adherence.**

Instruction

Renal transplantation is the best treatment of choice for patients with end-stage renal disease. In order to prevent the body from rejecting the graft, the use of immunosuppressive agents is very important. In recent decades, huge steps have been taken in the development of new immunosuppressive drugs, such as drugs with reduced nephrotoxic side effects. However, graft loss still occurs [14]. Mycophenolic acid, calcineurin inhibitors and mTOR-inhibitors are the most frequently used immunosuppressive agents in Europe. Transplant patients need to take these drugs for the rest of their lives [15]. Immunosuppressive agents have many unpleasant side effects, including increased risk of malignity, nephrotoxicity and weight gain. This makes non-adherence to immunosuppressants more easily justifiable and more frequent [1]. In our study we used the definition of medication adherence that is used in Pubmed: "Voluntary cooperation of the patient in taking drugs or medicine as prescribed. This includes timing, dosage, and frequency."

Because of the importance of patient adherence, many studies have been published on the effects of immunosuppressant non-adherence on graft failure in renal transplant patients.

Most of these studies examined predictors and prevention of non-adherence to immunosuppressive drugs. In 2004, a systematic review was published discussing the consequences of non-adherence, using articles released until 2001. Since then many developments have occurred [2]. For example, the impact of non-adherence to current immunosuppressive agents regimens is unknown. Therefore we decided to review all literature after 2001 on this subject. Because donor organs are scarce, it is important to gain knowledge about the effect of non-adherence on graft survival.

In our systematic review we addressed the following research question: what is the effect of non-adherence to current immunosuppressive agents on acute rejection, chronic rejection, graft failure, graft survival and allograft loss?

Methods

Search strategy

Table 1.1 - Predetermined exclusion criteria used by reviewers

Exclusion criteria
No articles about transplantations of the heart, liver, pancreas, lung,
bowel or tissues other than the kidney.
No articles published before January 2001.
No articles found in any other database than Pubmed.
No articles describing the factors associated with medication
adherence.
No tertiary publications and systematic reviews.
Articles which could not be obtained through Erasmus University
Rotterdam were excluded.

Table 1.2 - Predetermined inclusion criteria used by reviewers

Inclusion criteria
Study should be a cohort study or a randomized control trial
Participants of any age who received a renal transplantation
Participants who use one or more immunosuppressive agents
Graft failure, graft loss, graft survival or graft rejection as main outcome
Articles should concern the degree of non-adherence

To find all the relevant articles published on our topic, we searched the Pubmed database. We searched for articles published from 1 January 2001 to 18 January 2012.
We used the MeSH terms “Kidney Transplantation”, “Immunosuppressive agents”, “Patient Compliance”, “Medication Adherence”, “Treatment Refusal”, “Graft survival” and “Graft Rejection”. We only included English articles and excluded all reviews. Two reviewers read the titles and abstracts and used predetermined exclusion criteria (Table 1.1) to eliminate studies. The articles were checked on inclusion criteria by extensive reading (Table 1.2). We used an independent third reviewer to resolve disagreements between the other two reviewers.

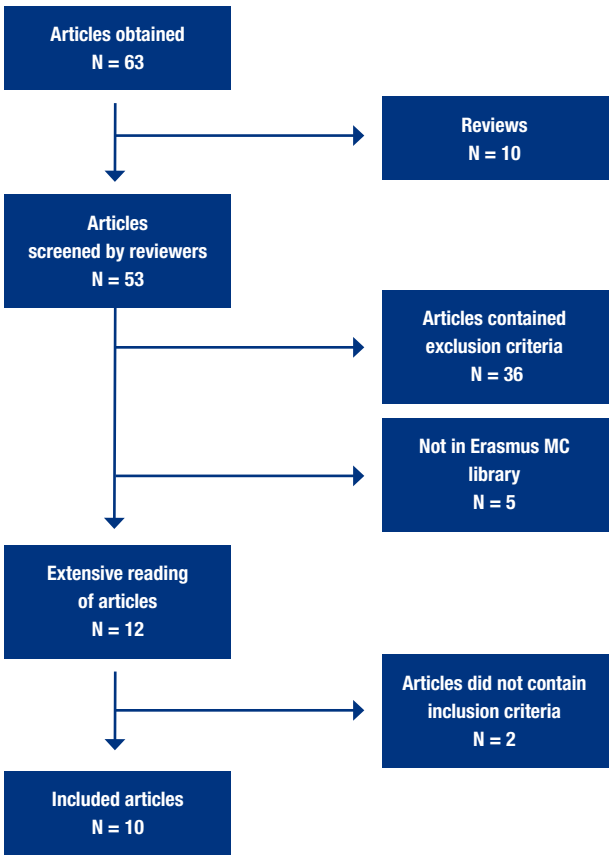
Data analysis

We extracted information from the articles about the authors, publication date, the follow-up duration and study population. We determined how the trial defined the degree of adherence and which patient groups the trial distinguished. Furthermore we noted the measure of outcome of the studies and the results that were relevant to our review objective.

Figure 1 -
Flow chart of study selection process.

Search in Pubmed database Limits: ‘English’ and ‘1 Jan. 2001- 18 Jan. 2012’

“Kidney Transplantation” [MeSH] AND “Immunosuppressive Agents” [MeSH] AND (“Patient Compliance” [MeSH] OR “Medication Adherence [MeSH] OR “Treatment Refusal” [MeSH]) AND (“Graft Rejection” [MeSH] OR “Graft Survival” [MeSH])



Quality Analysis

We assessed the risk of bias of the various trials, using the STROBE statement [13]. The STROBE statement, published in BMJ, is a checklist of items an observational study should contain. It focuses on transparency and completeness of the provided information. By determining whether the articles contained these items, we were able to identify and interpret the quality of the results.

Two reviewers evaluated the articles based on study type, population size, outcome measurement, data collection, way of grouping quantitative variables and reliability. We preferred prospective cohort studies over retrospective studies and assessed a better quality to large study groups. In prospective studies the criteria are determined before the study starts. By using prospective studies the chances of selection bias are reduced. The determination of the level of non-adherence to immunosuppressive agents was found more reliable when biopsy and serum creatinine levels were used in contrast to the use of interviews and questionnaires. Biopsy and serum creatinine levels do not depend on the memory and integrity of the participants.

Several methods are used to measure adherence. MEMS or EDEMs are electronic medicine bottles that register the time that the bottle or cap with the medication is opened. The shortcoming of this method is that patients can open the bottle without taking the medicine. Adherence can also be measured by determining serum level of the immunosuppressive agent. However, this level, can be influenced by diarrhea, food intake and other medicines. The MPR (medication possession rate), is calculated by determining the difference between the date that patients were expected to pick up their medication at the pharmacy and the date they actually picked it up. With this method patients can pick up their medicine at the right time, but still not take the medication. Finally interviews and questionnaires can be used to estimate adherence. However bias can easily occur, because of variance in interviewer, type of questionnaire, motive of the patient and memory. We decided that a combination of all these methods and correction for the dependent variables was qualitatively best.

We examined the extensiveness, overall reliability and completeness of the statistical information and determined if the participants were classified in different adherence groups.

Results

Study selection

After our initial search in Pubmed, we found 63 articles, of which 10 articles were excluded because they were reviews. After reading the abstracts we excluded a total of 41 articles. Because they did not investigate patient adherence, 6 additional articles were excluded

A total of 27 articles discussed outcome variables that did not match our review objective and we could not obtain the full text of 5 articles.

Furthermore, 1 case report and 2 editorial comments were excluded. After extensive reading of the articles we eliminated another 2 articles. We therefore included a total of 10 articles in our review.

Study characteristics

We summarized several study characteristics of the articles in Table 2. We also conducted a quality analysis of all articles by evaluating several validity criteria to assess risk of bias. The results are shown in Table 3. We used – and + to score the articles, where - - is the lowest score and ++ is the highest score an article could earn for a study characteristic. The quality of articles varied widely, although most articles were rated as good. Overall, the weakest factor was the type of study.

A randomized controlled trial setting is impossible with this research topic; all studies were cohort studies. This is because people already belong to a group 'non-adherent patients' or 'adherent patients'. You can't force patients to be non-adherent or adherent. Therefore all studies were cohort studies.

Furthermore, we found that some articles lacked an extensive description of the following aspects: the definition of non-adherence, methods and the calculation of the outcome.

Acute rejection

In 6 out of 10 articles we used, acute rejection as a result of non-compliance or non-adherence was studied. The prospective cohort study from Ghods et al. studied compliance vs. non-compliance (acute rejection $p<0.001$).

Shaw et al. (2003) described the relationship of non-adherence and adherence with acute rejection ($\chi2 = 46.4$, $p = 0.000$) and the role of non-adherence as a predictor of acute rejection (OR 6.91, $p = 0.001$). The prospective cohort study van Nevins et al. (2001) showed a late acute rejection when comparing declining compliance with steady compliance (OR 13.9 (95% CI 2.9 – 68), $p = 0.0011$). Israni et al. (2001) compared adherence level between 59% and 85% (the article, table 2 notes 59%- 85%), with adherence level above 85% up to 100% (>85%- 100%) for acute rejection. They reported a hazard ratio (HR) of 1.47, with a confidence interval of 95% (0.56-3.82). Adherence level less than 50% (<50%) and adherence level above 85% up to 100% (>85%-100%) were also compared. They reported a hazard ratio (HR) of 2.06, with a confidence interval of 95% (0.74-5.3).

Nevins and Thomas (2001) compared declining and steady azathioprine adherence. They found that acute rejection, graft loss before death or any adverse clinical outcome all increased with increasing non-compliance.

However, when groups with overall missed dose rates of less than 5% were compared with groups above 5%, no significant association was found with adverse outcomes. In a retrospective cohort study, Pinsky et al. (2009) examined acute rejection in relation to poor compliance and excellent compliance; they reported an acute rejection rate of 26.0% in the poor compliance group and 20.5% in the excellent compliance group ($p < 0.001$).

Chronic rejection

Of the 10 included articles, 2 showed a correlation with chronic rejection. Shaw et al. (2003) compared adherence to non-adherence (chronic rejection $\chi2 = 37.2$, $p = 0.000$) and non-adherence as a predictor (OR 4.98, p=0.004). Nevins and Thomas (2001) compared declining adherence with steady adherence (5.1 (SD 1.8), $p = 0.034$)).

Hsiau et al. studied allograft rejection (in general) compared to no rejection for serum tacrolimus (OR 9.7, $p = 0.005$) and MPA levels. Ghods et al. (2003) showed an advanced allograft dysfunction ($p<0.01$) in the study population with major non-compliance. Other results were not reported.

Graft survival and death

Only Chisholm-Burns et al. (2003) showed graft survival by comparing the adherent group with the non-adherent group: $\chi2 = 5.68$, $p = 0.017$.

Two studies reported results on death. Nevins and Thomas (2001) compared declining vs. steady adherence and reported a late patient death rate of 4.9% (SD 1.5, $p = 0.066$). Pinsky et al. (2009) examined the hazard ratio (HR) for patient death. They reported a HR of 1,24 with a confidence interval (CI) of 95% (0.95-1,64). Patients died 1,24 times more per unit time than the population with no poor compliance ($p = 0,1166$ for poor compliance).

Allograft loss

Allograft loss as a major outcome was used in 4 of the 10 articles. Shaw et al. (2003) found a relationship between graft loss and non-adherence to immunosuppressive agents ($\chi 2$ = 20.6, p = 0.000). They also examined whether non-adherence could be a predictor of graft loss, but found no positive relationship (OR 5.40, p= 0.07). Michelon et al. (2002) studied the 5-year graft loss rate. They found a compliance of 35.5% and noncompliance of 80.9%. No statistical test was performed. Nevins et al. (2001) found an association between graft loss and decline in compliance (OR 4.3, 95% CI 1.1-16; p=0.0321). Nevins and Thomas (2001) also examined the relationship between declining compliance and graft loss and reported an association (7.0 (SD 2.1), p <0.001) for patients with declined compliance. The number of participants was 7 and standard deviation was 2.1. Chisholm-Burns et al. (2003) studied the MPR quartile 4 (adherent group) vs. MPR quartile 1-3 (non-adherent group), in which they found $\chi 2 = 5.22$, p = 0.022 for graft failure, OR=2.07 (95% CI 1.12 – 4.06), p = < 0.05. The retrospective study from Takemoto et al. (2007) compared poor compliance with high compliance. For graft failure they reported HR (95% CI) 1.43 (1.11 – 1.84), p = 0.005. Pinsky et al. (2009) compared poor compliance to excellent compliance (graft failure HR (95% CI) 1.80 (1.52 – 2.13), p<0.0001).

Table 2 - Study characteristics

	Author	Study design	Number of patients	Follow up duration	Age of the study population	Comparison	Percentage Non-adherence	Outcome
1	Ghods et al. (2003)	Prospective cohort study	286	5-231 months (mean 76.7 SD 53.5)	12-70 (mean 39.1 SD 11.6) years	Compliance vs. noncompliance major	- 24.5%	Acute rejection, Advanced allograft dysfunction
2	Shaw et al. (2003)	Retrospective cohort study	112	Not reported	15.2 (SD 6.5) years	Non adherence vs. adherence	32.5%	Relation Chronic rejection Acute rejection Loss of graft Predictor Chronic rejection Acute rejection Loss of graft
3	Michelon et al. (2002)	Retrospective cohort study	1027	≥ 12 months	32.7 (SD 14.6) years	Compliance vs. non-compliance	Not reported	5-years graft loss rate
4	Nevins et al. (2001)	Prospective cohort study	180	50 (SD 12) months	42 (SD 13.7) years	Declining compliance vs. Steady compliance	11%	Late acute rejection graft loss
5	Israni et al. (2011)	Prospective cohort study	243	36 months	Recipients age ≥18	(a) Adherence Level (AL) 59- 85% vs. AL >85- 100% (b) AL < 50% vs. AL >85-100%	50-85% adherence = 20% < 50% adherence = 12%	Acute rejection
6	Chisholm-Burns et al. (2003)	Retrospective cohort study	877	≥3 months	Renal Transplant Recipient (RTR) age ≤18	Medication Possession Ratio (MPR) quartile 4 (adherent group) vs. MPR quartile 1- 3 (non-adherent group)	73%	Graft failure Graft survival
7	Hsiau et al. (2011)	Retrospective cohort study	46	4.3 SD 1.3 years	No rejection	Rejection vs. No rejection	Not reported	Allograft rejection

Quality analysis

The results of the quality analysis are shown in Table 3.

Table 3 - Quality analysis of all articles based on evaluation of several study characteristics

	Study type	Study size	Outcome measurement	Data collection	Grouping quantitative variables	Reliability	Extensiveness of Statistics	Total
Ghods et al.	+	+	-	--	+	-	-	--
Shaw et al.	-	+	+	+	+	+	+	+++++
Michelon et al.	-	++	-	--	+	-	+	-
Nevins et al.	+	+	+	++	-	+	+	++++++
Israni et al.	+	+	+	++	++	+	+	+++++++++
Chisholm-Burns et al.	-	++	-	-	++	+	+	+++
Hsiau et al.	-	-	+	+	-	-	+	-
Nevis and Thomas et al.	+	+	+	++	++	+	+	+++++++++
Takemoto et. al	-	++	-	-	+	+	+	++
Pinksy et al.	-	++	-	-	++	-	+	+

Good quality (+)
Poor quality (-)
Very good quality (++)
Very poor quality (--)

Table 4 - summary of individual study outcomes

	Author	Comparisson	Result
Acute rejection	Ghods et al. (2003)	Compliance vs. noncompliance major	Acute rejection episode and advance allograft dysfunction (P <0.001 and P<0.01)
	Shaw et al. (2003)	Non adherence vs. adherence	Non- adherence (NA) in the complete sample (x2= = 46.4, p = 0.000)
			NA as predictor of acute rejection (OR 6.91, p =0.0001)
	Nevins et al. (2001)	Declining compliance vs. Steady compliance	Late acute rejection (OR 13.9 (95% CI 2.9 - 68), p = 0.0011)
	Israni et al. (2011)	(a) Adherence Level (AL) 59- 85% vs. AL >85- 100% (b) AL < 50% vs. AL >85-100%	(a) (HR (95% CI)1.47 (0.56 - 3.82)) (b) (HR (95% CI) 2.06 (0.74 – 5.73))
	Nevins and Thomas (2001)	Declining vs. Steady	Late acute rejection rate 9.6% (SD 2.5), p <0.001
	Pinsky et al. (2009)	Poor compliance vs. excellent compliance	26.0% vs. 20.5%, p <0.001
Chronic rejection	Shaw et al. (2003)	Non adherence vs. adherence	Non- adherence (NA) in the complete sample (x2= 37.2, p = 0.000)
			NA predictor as a predictor of chronic rejection (OR 4.98, p = 0.004)
			Chronic rejection rate (SD 5.1), p = 0.034
General rejection	Nevins and Thomas (2001)	Declining vs. Steady	OR 9.7, p = 0.005
	Hsiau et al. (2011)	Rejection vs. No rejection for Tacrolimus (TAC) vs. Mycophenolic acid (MPA)	x2=5.22, (p = 0.022)
Graft failure	Chisholm-Burns et al. (2003)	Medication Possession Ratio (MPR) quartile 4 (adherent group) vs. MPR quartile 1- 3 (non-adherent group)	OR= 2.07, 95% CI 1.12 – 4.06, p< 0.05
			HR (95% CI) 1.43 (1.11 – 1.84), p = 0.005
	Takemoto et al. (2007)	Poor compliance vs. High compliances	HR (95% CI) 1.80 (1.52 – 2.13), p <0.0001
	Pinksy et al. (2009)	Poor compliance vs. excellent compliance	Difference in graft survival between adherence and non-adherence group (x2=5.68, p = 0.017)
Graft survival	Chisholm-Burns et al. (2003)	Medication Possession Ratio (MPR) quartile 4 (adherent group) vs. MPR quartile 1- 3 (non-adherent group)	Non- adherence in the complete sample (x2= 20.6, p = 0.000)
Graft loss	Shaw et al. (2003)	Non adherence vs. adherence	Non- adherence (NA) is not a predictor of graft loss (OR 5.40, p = 0.07)
			35.5% compliance, 80.9% non-compliance
	Michelon et al. (2002)	Compliance vs. non-compliance	OR (95% CI) of graft loss were 4.3 (1.1 – 16, p= 0.0321)
	Nevins et al. (2001)	Declining compliance vs. Steady compliance	times higher in patients with declining compliance vs. the steady compliers
			Graft loss before death rate 7.0 (SD 2.1), p <0.001
	Nevins and Thomas (2001)	Declining vs. Steady	Late Death rate 4.9 (SD 1.5), p = 0.066
Death	Nevins and Thomas (2001)	Declining vs. Steady	HR (95% CI) 1.24 (0.95 – 1.64, p = 0.1166)
	Pinsky et. al	Poor compliance vs. excellent compliance	Transplant recipients with major non- compliance had more
Graft dysfunction	Ghods et al. (2003)	Compliance vs. noncompliance major	allograft dysfunction (p <0.01)

Discussion

Because of the scarce supply of donor organs it is important to gain knowledge about the effect of medication non-adherence on graft survival. In this review we collected information about acute rejection, chronic rejection, graft failure, allograft loss and decreases graft survival.

Our conclusion is that the effect of non-adherence to current immunosuppressive agents, is that non-adherence increases the likelihood of acute rejection, chronic rejection, graft failure and graft loss. Non-adherence might decrease the graft survival.

Limitations

Our study was limited by certain factors. Although the overall quality of the included studies was good, there was some variation between the different studies. Of the 19 included articles, 3 had questionable reliability [1,3,7]. The overall weakness of most of the studies was the study type, as most articles used a retrospective study [2,3,6,7,9,10]. In addition, many methods were used to determine adherence. Questionnaires and interviews, which are less accurate methods, were used in 5 studies [1,3,6,9,10]. This also led to different definitions of adherence, which made comparisons less accurate. However, we did not find any differences in results between the reliable and less reliable studies.

Conclusion

We can conclude that substantial improvement of adherence is expected to improve graft survival. Obviously, this knowledge alone would not improve graft survival. The main goal for future research should be to study how adherence can be improved. In this way protocols can be established to minimize non-adherence and thus reduce graft rejection.

Several groups at risk for non-adherence have been previously defined. For example, negroid people have a higher non-adherence rate [5]. A challenge will be to identify more such groups, find a way to enhance their adherence, for example through regular monitoring by their physicians and improving education about the effect of non-adherence after transplantation.

References

1. *Ghods AJ, Nasrollahzadeh D. Noncompliance with immunnosuppressive medications after renal transplantation. Experimental and clinical transplantation 2003; 1: 39-47.*
2. *Shaw RJ, Palmer L, Blasey C, Sarwal M. A typology of non-adherence in pediatric renal transplant recipients. Pediatric Transplantation 2003; 7: 489-493.*
3. *Michelon TF, Piovesan F, Pozza R, Castilho C, Bittar AE, Keitel E, Santos A, Goldani JC, Garcia CD, Neumann J, Garcia VD. Noncompliance as a cause of renal graft loss. Transplantation Proceedings 2002; 34: 2768-2770.*
4. *Nevins TE, Kruse L, Skeans MA, Thomas W. The natural history of azathioprine compliance after renal transplantation. Kidney International 2001; 60: 1565-1570.*
5. *Israni AK, Weng FL, Cen YY, Joffe M, Kamoun M, Feldman HI. Electronically measured adherence to immunosuppressive medications and kidney function after deceased donor kidney transplantation. Clinical Transplantation 2011; 25: 124-131.*
6. *Chisholm-Burns MA, Spivey CA, Rehfeld R, Zawaideh M, Roe DJ,Gruessner R. Immunosuppressant therapy adherence and graft failure among pediatric renal transplant recipients. American Journal of Transplantation 2009; 9: 2497-2504.*
7. *Hsiau M, Fernandez HE, Gjertson D, Ettenger RB, Tsai EW. Monitoring nonadherence and acute rejection with variation in blood immunosuppressant levels in pediatric renal transplantation. Transplantation 2011; 92: 918-922.*
8. *Nevins TE, Thomas W. Quantitative patterns of azathioprine adherence after renal transplantation. Transplantation 2009; 87: 711-718.*
9. *Takemoto SK, Pinsky BW, Schnitzler MA, Lentine KL, Willoughby LM, Burroughs TE, Bunnapradist S. A retrospective analysis of immunosuppression compliance, dose reduction and discontinuation in kidney transplant recipients. American Journal of Transplantation 2007; 7: 2704-11.*
10. *Pinksy BW, Takemoto SK, Lentine KL, Burroughs TE, Schnitzler MA, Salvalaggio. Transplant outcomes and economic costs associated with patient noncompliance to immunosuppression. American Journal of Transplantation 2009; 9; 2597-2606.*
11. *Butler JA, Roderick P, Mullee M, Mason JC, Peveler RC. Frequency and impact of nonadherence to after renal transplantation: a systematic review. Transplantation 2004; 77: 769-789.*
12. *Pollock-Barziv SM, Finkelstein Y, Manlhiot C, Dipchand AI, Hebert D, Ng VL, Solomon M, McCrindle BW, Grant D. Variability in tacrolimus blood levels increases the risk of late rejection and graft loss after solid organ transplantation in older children. Pediatric Transplantation 2010; 14: 968-975.*
13. *Von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP.*
    Strenghtening the reporting of observational studies in the epidemiology (STROBE) statement: guidelines for reporting observational studies. BMJ 2007; 335: 806-808.
14. *Cooper JE, Wiseman AC. Novel immunosuppressive agents in kidney transplantation. Clinical Nephrology 2010; 73: 333-343.*
15. *Van Gelder T. European Society for Organ Transplantation Advisory Committee recommendations on generic substitution of immunosuppressive drugs. Transplant International 2011; 24: 1135-1141.*

Systematic review

Natriuretic peptide guided therapy versus clinically guided therapy in patients with chronic heart failure

A systematic review of randomized controlled trials

Bart van Tunen [a], Fei Fan Choi [a], Jin Ming Cheng [b]
[a] Medical student, Erasmus MC University Medical Center Rotterdam, the Netherlands
[b] Supervisor, Department of Cardiology, Erasmus MC University Medical Center Rotterdam, the Netherlands
Correspondence: Bart van Tunen, e-mail: 332645bt@student.eur.nl

Background: **Plasma levels of B-type natriuretic peptide (BNP) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) are established indicators of decompensated heart failure (HF) and predictors of heart failure morbidity and mortality. These natriuretic peptides (NPs) may serve as a useful tool in guiding the therapy of patients. However, when comparing NP-guided therapy with clinically guided therapy, it remains unclear which treatment gives better outcomes. In this review, our aim is to clarify whether NP-guided HF treatment reduces morbidity and/or mortality when compared with clinically guided therapy.**
Methods: **Using PubMed, we identified all randomized controlled trials (RCTs) indexed for MEDLINE before 21 January 2012, comparing BNP/NT-proBNP-guided therapy with clinically guided therapy for chronic heart failure (CHF). The reference lists of the articles identified were checked for additional publications. In total we examined nine studies for this review.**
Results: **The studies we examined seemed to show that patients who were treated with NP-guided therapy tend to benefit when it came to all-cause mortality, hospital-free survival and cardiovascular events compared to those who received clinically guided therapy, although most results were not statistically significant. In addition, patients ≤ 75 years of age seemed to benefit more from NP-guided therapy than patients > 75 years.**
Conclusion: **The benefits of NP-guided therapy were not always significantly proven, nor were they consistent across all the trials. Therefore, we conclude, that it is too early to replace existing therapy for CHF patients with NP-guided therapy.**

Introduction

Heart failure (HF) is a common clinical syndrome representing the end-stage of a number of different cardiac diseases. In recent years, the prevalence of HF in the western world has continued to increase [1]. Chronic heart failure (CHF) remains the main reason for hospital stay among patients aged 65 years or older and is associated with high mortality (average, overall case fatality rate per year: 17.1% and 15.1% for incident and prevalent cases) [2]. The rising incidence and prevalence of CHF constitutes a major health care burden [3].

The goals of HF therapy are clinical a reduction in risk of morbidity and mortality of HF patients, followed by a stabilization of their condition. Current management, also called clinically guided therapy, of patients with HF is mainly based on clinical signs and symptoms [4]. This approach allows clinicians to respond to worsening HF once it is recognized, but does not allow selection of individuals who are most likely to progress to increased morbidity and mortality and are thus in need of more intensive treatment [4]. Moreover, even with intensive treatment, mortality and morbidity are high [5]. This may be attributed to suboptimal care guided by clinical signs and symptoms [6].

Elevated plasma levels of B-type natriuretic peptide (BNP) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) are established indicators of decompensated HF and predictors of HF morbidity and mortality [4]. These natriuretic peptides (NPs) are highly correlated to left ventricular filling pressure and may serve as a useful tool in guiding the medical treatment of patients [1]. If clinically guided therapy is replaced by NP-guided therapy, morbidity and/or mortality might improve.

However, it remains unclear which treatment is superior. We therefore systematically reviewed the literature to clarify whether NP-guided HF treatment improves any of the outcomes of total mortality, hospital-free survival or total cardiovascular events, when compared with clinically guided therapy.

Methods

Using PubMed, we identified all randomized controlled trials (RCTs) indexed for MEDLINE before 21 January 2012, comparing BNP/NT-proBNP-guided therapy with clinically guided therapy for CHF. All qualifying studies were assessed for patient characteristics and outcomes. The reference lists of the articles identified were checked for additional publications. For this review, all heart failure patients were included.

Each search query used RCT as a limit and included the major MeSH term “Natriuretic Peptide, Brain/blood”, AND the MeSH term “Cardiac Output, Low/drug therapy” OR “Heart Failure/therapy” OR “Heart Failure/drug therapy” AND the MeSH term “Angiotensin II Type 1 Receptor Blockers” OR “Angiotensin-Converting Enzyme Inhibitors” OR “ambulatory care”.

Of the studies found, only those articles that specifically compared the effectiveness of BNP/NT-proBNP-guided therapy with clinically guided therapy or equivalent were considered. Studies also had to contain one or more of the following end points to be included in this review: all-cause mortality, survival free of any hospitalization, total cardiovascular events and HF-related hospitalization. These outcomes, together with their age-group subdivision (if used), were compared, in order to ascertain which therapy, BNP/NT-proBNP-guided therapy or clinically guided therapy, is most effective for treatment of patients with CHF.

Results

Using our search protocol, we identified 8 articles. The reference lists of the articles identified were checked for additional publications. Doing this, we found another important recently published article that has not been indexed in MEDLINE yet [7]. In total we examined 9 articles for our review (Table 1).

Total mortality

The first endpoint we assessed was total mortality. This outcome was used in 6 studies. Of those, only the BATTLESCARRED [8] trial could prove any difference between NP-guided therapy and UC.

In the BATTLESCARRED [8] trial, treatment strategies were applied for 2 years with follow-up to 3 years. Mortality at 1 year was 9.1% in the NT-proBNP group and 18.9% in UC patients ($p = 0.028$). Univariate Kaplan-Meier analysis showed no overall difference in survival among groups at 2 and 3 years, but on Cox regression, an overall treatment effect spanned the 3 years ($p = 0.033$), driven by a significant difference between the NT-proBNP and UC group ($p = 0.011$).

In the UPSTEP [1] trial, there was no significant difference in all-cause mortality between groups. Even after univariate Cox proportional regression analysis, no significant difference in risk of the primary outcome variable (a composite of death from any cause, need for hospitalization and worsening heart failure) was found between the BNP group and the UC group.

Table 1 - Studies included in this review

Trial name or authors name	n	Inclusion criteria	Natriuretic peptide target	Control group(s)	Follow-up	Primary endpoint(s)
UPSTEP(1)	279	NYHA class II–IV and/or symptoms of worsening HF LVEF < 40% BNP > 150 ng/l if < 75 years, and > 300 ng/l for those aged > 75 years	BNP levels to < 150 ng/l if < 75 years old or < 300 ng/l in if ≥ 75 years old	Conventional HF treatment according to guidelines	12 months	Total mortality, all-cause hospitalization and worsening HF
STARS-BNP(2)	220	Stable outpatients Optimal background therapy NYHA class II-III LVEF < 45%	BNP < 100 ng/l for first 3 months after randomization	Clinical judgment	15 months	Unplanned HF hospitalization or HF death
PROTECT(3)	151	HF due left ventricular systolic dysfunction LVEF ≤ 40%	NT-proBNP concentrations ≤ 1,000 pg/ml	Standard of care management	10 months	Total cardiovascular events
PRIMA(4)	345	Hospitalized with HF Preserved or reduced LVEF NT-proBNP > 1,700 ng/l at hospital admission NT-proBNP drop by > 10% before hospital discharge	NT-proBNP at discharge or at 2 weeks' follow-up	Clinical judgment	1.9 years (median)	Hospitalization free survival
The Christchurch New Zealand pilot trial(5)	69	NYHA class III-IV LVEF < 40%	NT-proBNP < 1,691 ng/l	Clinical judgment	9.5 months (median)	Cardiovascular death or hospitalization
Berger et al.(6)	278	Signs of cardiac decompensation NYHA class III-VI Cardiothoracic ratio > 0.5 or LVEF < 40%	NT-proBNP < 2,200 pg/ml	Multidisciplinary care or usual care	18 months	HF rehospitalization, time to combined end point of death and HF rehospitalization, first HF rehospitalization, and death
STARBRITE(7)	130	NYHA class III–IV LVEF ≤ 35%	BNP < 2x hospital discharge	Standardized congestion score	90 days	Hospitalization free survival
BATTLESCARRED(8)	364	Symptomatic HF with preserved or reduced LVEF Recent hospitalization with HF (< 2 weeks) NT-proBNP > 400 ng/l	NT-proBNP < 1,300 ng/l	Standardized HF score or standard care	2.8 years (median)	Total mortality or HF hospitalization
TIME-CHF(9)	499	Age ≥ 60 years NYHA class II–IV LVEF ≤ 45% Hospitalized with HF in past year NT-proBNP > 2x upper limit of normal	NT-proBNP < 400 ng/l if < 75 years old or < 800 ng/l if ≥ 75 years old	Clinical judgment	18 months	Hospitalization free survival and quality of life

In the PRIMA [4] trial, mortality was lower in the NT-proBNP–guided group compared to the clinically guided group, but this was not statistically significant.

In the TIME-CHF [9] trial, all-cause mortality rates did not differ significantly.

In the Christchurch New Zealand [5] pilot trial, one patient died suddenly in the BNP group (n = 33) and seven in the clinical group (n = 36), but this was not statistically significant.

In the STARS-BNP [2] trial, all cause death was not significantly different between the groups.

Age-group subdivision

The BATTLESCARRED [8] trial observed interactions (p = 0.025) between treatment group and age with respect to mortality. At 1, 2, and 3 years among patients ≤ 75 years of age, cumulative all-cause mortality was 1.7%, 7.3%, and 15.5%, respectively, in the NT-proBNP group; and 20.3%, 23.4%, and 31.3%, respectively, in the UC group. Three-year mortality was significantly lower for the younger NT-proBNP patients than for their peers in the UC group (p = 0.021), whether analysis included all patients randomized (p = 0.03) or was confined to subjects with follow-up of at least 3 years (n = 215) from randomization (p = 0.008).

For the prespecified subgroups in the UPSTEP [1] trial, age ≤ 75 years vs. age > 75 years, we found no significant differences in risk for all-cause mortality.

Hospital-free survival

The second endpoint we assessed was survival free of any hospitalization. This outcome was used in 4 studies. Here too, only the BATTLESCARRED [8] trial could prove any difference between NP-guided therapy and UC.

In the PRIMA [8] trial, management guided by an individualized NT-proBNP target did not significantly improve their primary end point: median number of days alive outside the hospital was 685 versus 664 days.

In the TIME-CHF [9], the NT-proBNP–guided strategy did not improve 18-month survival free of any hospitalization (41% for NT-proBNP–guided group vs. 40% for symptom-guided group).

In the STARBRITE [7] trial, the mean number of days alive, not hospitalized, and without left ventricular assist device (LVAD) or transplant was 85 ± 12.1 days in the BNP strategy and 80.4 ± 20.6 days in the congestion score strategy. There was no significant difference between the groups.

Age-group subdivision

In the BATTLESCARRED [8] trial, a comparison of "days alive and not in hospital with heart failure" for patients with potential follow-up of 1, 2, and 3 years favored patients ≤ 75 years in the NT-proBNP group: totals at 1, 2, and 3 years averaged 360, 690, and 1,012 days, respectively, in the younger NT-proBNP patients; and 318, 584, and 806 days, respectively, for patients receiving UC. The difference between the NT-proBNP and UC groups in this regard was sustained (p = 0.04, p = 0.07, and p = 0.04, respectively, at 1, 2, and 3 years). On average over 3 years among younger patients, the NT-proBNP group had 206 more days (of a possible 1,076) alive and not in hospital with heart failure than did the UC group (p = 0.04). No significant benefit was observed from NT-proBNP over UC among patients > 75 years of age.

Total cardiovascular events

The final end point we were interested in, was the total number of cardiovascular events. Two studies used this outcome, both showing a significant smaller number of events in the NP-guided therapy compared to UC.

The Christchurch New Zealand [5] pilot trial showed there were fewer total cardiovascular events (death, hospital admission, or heart failure decompensation) in the BNP group than in the clinical group (19 vs. 54, p = 0.02) during the median 9,5 months of follow up. At 6 months, 27% of patients in the BNP group and 53% in the clinical group had experienced a first cardiovascular event (p = 0.034).

Through a mean follow-up period of 10 ± 3 months, the PROTECT [3] study showed a significant reduction in the primary end point of total cardiovascular events (worsening HF, hospitalization for HF, clinically significant ventricular arrhythmia, acute coronary syndromes, cerebral ischemia, and cardiac death) in the NT-proBNP arm compared with standard of care arm (58 events vs. 100 events, p = 0.009; logistic odds for events 0.44, p = 0.02).

HF-related hospitalization

As a sub point, we also looked at HF-related hospitalization. Of the 5 studies containing this outcome, only Berger et al. [6] and the STARS-BNP [2] trial could prove a difference between NP-guided therapy and UC.

Berger et al. [6] showed, by using Kaplan-Meier analysis, that the first HF rehospitalization (28%) was lower in the NT-proBNP–guided, intensive patient management group (BM) than in the multidisciplinary care (MC) group (40%; p = 0.06), and in the MC versus UC group (61%; p = 0.01). It should be noted that the BM group consisted out of NP-guided therapy and multidisciplinary care combined.

In the STARS-BNP [2] trial, hospital stays for HF were observed in 22 patients in the BNP group versus 48 patients in the clinical group (p = 0.0001).

In the PRIMA [4] trial, the total number of cardiovascular and HF-related admissions between the NT-proBNP–guided and the clinically guided groups were not different.

In the BATTLESCARRED [8] trial, hospitalizations for HF did not differ among groups (cumulative event rates over 3 years of 36% and 34% for NT-proBNP and UC groups, respectively).

In the UPSTEP [1] trial, time to first HF hospitalization did not differ significantly.

Age-group subdivision

In the BATTLESCARRED [8] trial, a non-significant reduction for HF hospitalization was observed among younger NT-proBNP patients (29% vs. 36%).

For the prespecified subgroups in the UPSTEP [1] trial, a non-significant reduction in primary outcome events (composite of death due to any cause, need for hospitalization and worsening HF) could also be found. In the group ≤ 75 years there were 45 primary outcome events in the BNP-group (82 patients) compared to 51 events in the UC group (81 patients). For the group > 75 years there were 40 primary outcome events in the BNP-group (58 patients) compared to 37 events in the UC group (47 patients).

Discussion

Our study suggest that patients receiving NP-guided therapy tend to benefit when it comes to all-cause mortality, survival free of any hospitalization, total cardiovascular events and HF-related hospitalization. These benefits however were not always significantly proven, nor were they consistent across all the trials (Table 2).

When looking at the age-group subdivision, the group ≤ 75 years seemed to do better than the group > 75 years. But here too, results were inconsistent and not always significantly proven.

Of the outcomes we examined, trials have only yielded consistent results when it comes to total cardiovascular events. Potential limitations of these studies, the Christchurch New

Zealand [5] pilot trial and the PROTECT [3] study, include small study size and the exclusion of difficult to treat population, such as those with heart failure with preserved ejection fraction (HFpEF).

Limitations

Despite our best efforts, comparing outcomes across existing trials remained difficult because of the variations in study populations, interventions, duration of follow-up and end points. For example, NP group targets are different among studies. Even now, no clear guidelines for NP-guided therapy exist. Despite these limitations, the trend suggests that NP-guided therapy could improve the clinical outcome of HF patients, especially for those ≤ 75 years.

Conclusion

Because of the inconsistent results, it is our belief that it is too early to replace existing therapy for CHF patients with NP-guided therapy. More well powered trials are needed to establish clear guidelines for NP-guided therapy and to ascertain the benefits of NP-guided therapy to the treatment of CHF.

Table 2 - Summary of results; + indicates a statistically improvement in outcome in the NP-guided-therapy group compared to clinically guided heart failure therapy, = indicates no proven difference

Trial name or authors name	Total mortality	Hospital-free survival	Total cardiovascular events	HF related hospitalization
UPSTEP(1)	=			=
STARS-BNP(2)	=	=		+
PROTECT(3)			+	
PRIMA(4)	=			=
Christchurch(5)			+	
Berger et al.(6)				+
STARBRITE(7)		=		
BATTLESCARRED(8)	+	+		=
TIME-CHF(9)	=	=		

References

1. *Karlstrom P, Alehagen U, Boman K, et al. Brain natriuretic peptide-guided treatment does not improve morbidity and mortality in extensively treated patients with chronic heart failure: responders to treatment have a significantly better outcome. European Journal of Heart Failure. 2011; 13:1096-103.*
2. *Jourdain P, Jondeau G, Funck F, et al. Plasma brain natriuretic peptide-guided therapy to improve outcome in heart failure: the STARS-BNP Multicenter Study. Journal of the American College of Cardiology. 2007; 49: 1733-9.*
3. *Januzzi JL, Jr., Rehman SU, Mohammed AA, et al. Use of amino-terminal pro-B-type natriuretic peptide to guide out- patient therapy of patients with chronic left ventricular systolic dysfunction. Journal of the American College of Cardiology. 2011; 58: 1881-9.*
4. *Eurlings LW, van Pol PE, Kok WE, et al. Management of chronic heart failure guided by individual N-terminal pro-B-type natriuretic peptide targets: results of the PRIMA (Can PRo-brain-natriuretic peptide guided therapy of chronic heart failure IM- prove heart failure morbidity and mortality?) study. Journal of the American College of Cardiology. 2010; 56: 2090-100.*
5. *Troughton RW, Frampton CM, Yandle TG, et al. Treatment of heart failure guided by plasma aminoterminal brain natriuretic peptide (N-BNP) concentrations. Lancet. 2000; 355: 1126-30.*
6. *Berger R, Moertl D, Peter S, et al. N- terminal pro-B-type natriuretic peptide-guided, intensive patient manage- ment in addition to multidisciplinary care in chronic heart failure a 3-arm, prospective, randomized pilot study. Journal of the American College of Cardiology. 2010; 55: 645-53.*
7. *Shah MR, Califf RM, Nohria A, et al. The STARBRITE trial: a randomized, pilot study of B-type natriuretic peptide- guided therapy in patients with advanced heart failure. Journal of Cardiac Failure. 2011; 17: 613-21.*
8. *Lainchbury JG, Troughton RW, Strangman KM, et al. N-terminal pro-B-type natriuretic peptide-guided treatment for chronic heart failure: results from the BATTLESCARRED (NT-proBNP- Assisted Treatment To Lessen Serial Cardiac Readmissions and Death) trial. Journal of the American College of Cardiology. 2009; 55: 53-60.*
9. *Pfisterer M, Buser P, Rickli H, et al. BNP-guided vs symptom-guided heart failure therapy: the Trial of Intensified vs Standard Medical Therapy in Elderly Patients With Congestive Heart Failure (TIME-CHF) randomized trial. JAMA: The Journal of the American Medical Association. 2009; 301: 383-92.*

Systematic review

The Efficacy and Safety of the HCV protease inhibitors telaprevir and boceprevir

A systematic review

Iris van Blitterswijk[a], Merel C. Broers[a], Lisanne M. Lawant[a], Bart Rijnders[b]
[a] Medical students, Erasmus MC University Medical Center Rotterdam, the Netherlands
[b] Supervisor, Department of Internal Medicine and Infectious Diseases, Erasmus MC University Medical Center Rotterdam, the Netherlands
Correspondence: Iris van Blitterswijk, e-mail: 341598ib@student.eur.nl

Objective: **To determine the efficacy and safety of the drugs telaprevir or boceprevir as treatment for Hepatitis C virus (HCV).**
Methods: **We searched Pubmed from January 13 to 17, 2012, using the major MeSH terms 'Hepacivirus' combined with 'Protease inhibitors' in our first search and the search terms 'telaprevir' or 'boceprevir' in our second search. Inclusion criteria were: HCV genotype 1, phase III study, efficacy measured by Sustained Virologic Response (SVR) and safety by rates of adverse events. Publications containing participants positive for Hepatitis B or HIV were excluded.**
Results: **Our searches yielded 21 publications, of which 4 publications met our inclusion criteria. Participants using boceprevir or telaprevir with peginterferon-ribavirin, compared to participants using peginterferon-ribavirin only – the current treatment of HCV –, showed a significantly higher SVR ($p<0.001$) and anemia was significantly more common ($p<0.001$) in boceprevir groups than in controls.**
Conclusions: **The addition of telaprevir or boceprevir to peginterferon-ribavirin results in a significantly higher SVR. Boceprevir groups more frequently report adverse events.**

Introduction

Worldwide about 130-170 million people are infected with hepatitis C virus (HCV) [1]. Many patients with chronic HCV die from HCV-related liver diseases, such as progressive hepatic fibrosis, cirrhosis and hepatocellular carcinoma [2]. The current standard treatment is the combination of peginterferon alfa and ribavirin [3,4]. Response to treatment is measured by the sustained virologic response (SVR), defined as undetectable plasma HCV RNA 24 weeks after completing treatment. However, this treatment leads to SVR in only 40 to 50% of non-previously treated chronic HCV genotype 1 patients [3-5]. Therefore, a new treatment concept is needed to increase the percentage of SVR in patients with HCV genotype 1.

One recent approach is the use of telaprevir and boceprevir. These are protease inhibitors that inhibit the NS3/4A serine protease, essential for viral replication, which results in lower HCV RNA levels [6]. In phase II studies, Telaprevir in combination with peginterferon-ribavirin was correlated with significantly higher response rates than the standard treatment [7-9].

Phase II studies have found antiviral effects of boceprevir in patients with HCV genotype 1. This effect was found both in previously untreated patients and in patients who received prior treatment [11]. Based on these findings, we decided to compare the efficacy and safety of the current standard treatment for HCV genotype 1 with the efficacy and safety of this standard treatment combined with telaprevir or boceprevir. In this systematic review we therefore addressed two questions. First, what is the efficacy of this combined treatment in patients with HCV genotype 1 compared with standard treatment, measured by rates of SVR? Secondly, what is the safety of combined treatment in patients with HCV genotype 1 compared to standard treatment, measured by rates of adverse events? We hypothesize 1) that treatment with peginterferon-ribavirin combined with protease inhibitor telaprevir or boceprevir results in a higher SVR rate than the standard treatment, and 2) that the rates of adverse events of this new treatment are higher.

Methods

We searched for randomized controlled clinical trials on the efficacy and safety of HCV protease inhibitors compared with standard HCV treatment on MEDLINE (OVID) electronic database (using PubMed). We combined the Medical Subject Headings (MeSH) terms "Hepacivirus" and "Protease inhibitors." Both MeSH terms had to be a major topic of the publication. Publications were limited to English, and the type of article had to be a randomized controlled trial. No limits were applied to date of publication or sex. We performed this search on January 13, 2012.

To increase the amount of relevant publications, we performed another search using the PubMed database. We used the search terms "telaprevir or boceprevir" and the limits "core clinical journals", "humans", "English" and "randomized controlled trial". We performed this search on January 17, 2012.

Additional inclusion criteria were (1) HCV genotype 1; (2) phase III study; (3) efficacy measured by SVR (in HCV RNA levels); (4) safety measured by rates of adverse events.

We excluded publications containing participants with a positive hepatitis B surface antigen and patients with positive antibodies to human immunodeficiency virus 1 and 2, as these aspects could influence the efficacy of the treatment of hepatitis C.

Assessment of efficacy

The primary outcome measure was the SVR in patient groups receiving a protease inhibitor and the standard treatment of peginterferon and ribavirin, compared to groups with standard treatment or standard treatment with a placebo. To analyze the data from the various studies, we compared the SVR of the various groups, thus indicating the efficacy of the therapy.

Table 1 - Treatment schedules of the different study groups

Publication	Study group	Telaprevir or Boceprevir use (wks)	Placebo* use (wks)	Peginterferon + ribavirin use (wks)	Total treatment Duration (wks)
Telaprevir not pre-treated	Group 1 (T0 + Placebo12 + PR36)	0	12	36	48
(Jacobson, et al., [13])	Group 2 (T8 + Placebo4 + PR12 or PR36)	8	4	12	24
				36	48
	Group 3 (T12 + PR12 or PR36)	12	0	12	24
				36	48
Boceprevir	Group 1 (PR4 + Placebo44)	0	44	4	48
pre- treated	Group 2 (PR4 + B32 (+ Placebo12))	32	0	4	36
(Bacon, et al., [14])			12		48
	Group 3 (PR4 + B44)	44	0	4	48
Boceprevir not	Group 1 (PR4 +Placebo44)	0	44	4	48
pre-treated (Poordad, et al.,	Group 2 (PR4 + B24 (+ Placebo20)	24	0	4	28
[15])			20		48
	Group 3 (PR 4 + B44)	44	0	4	48
Telaprevir not pre-treated	Group A (T12 + PR12)	12	0	12	24
(Sherman K, et al., [16])	Group B (T12 + PR36) (randomly)**	12	0	36	48
	Group C (T12 + PR36) (non-randomly)**	12	0	36	48

Each study group had a different treatment schedule, consisting of telaprevir (T), boceprevir (B), ribavirin (R), peginterferon (P) and/ or a placebo for boceprevir or telaprevir. Duration of treatment varied between 0, 4, 8, 12, 20, 24, 32, 36 or 44 weeks.
* Placebo were used for peginterferon and ribavirin.
** In Group C, participants were not randomly assigned to use peginterferon-ribavirin for 36, because assigning them to a 12 week schedule of peginterferon-ribavirin use would negatively influence their health (treatment duration would be too short). These included patients who had not experienced an extended rapid virologic response.

Assessment of safety

The safety of the treatment was measured by rates of adverse events, including anemia, rash, fatigue and gastrointestinal disorders (nausea and diarrhea). We determined the rate of occurrence for each adverse event in the various study groups.

Results

Our Pubmed search yielded 21 publications, of which 4 [13-16] met the inclusion criteria for our systematic review. The remaining publications (17) were excluded. Our complete study population consisted of 3.130 participants who received at least one dose of a relevant drug. Most studies were excluded because they contained participants positive for Hepatitis B or HIV. One publication lacked original data.

We compared the publications on efficacy and safety of protease inhibitors. Three of the publications [13-15] compared the use of a protease inhibitor (i.e. boceprevir or telaprevir) combined with standard therapy schedules using peginterferon alfa (subcutaneously) and ribavirin (oral) for the treatment of HCV, with the use of standard therapy schedules (peginterferon alfa and ribavirin) only.

One of the four publications [16] consisted of a non-inferiority study examining the efficacy of different schedules of telaprevir combined with peginteferon-ribavirin. Three publications [13,15,16] did not use pre-treated patients, and one publication [14] examined a previously treated group.

Efficacy

To examine the efficacy of protease inhibitors, we compared the results containing information about SVR, which in all trials was measured with the TaqMan 2.0 assay. We first compared the different treatment schedules used in the different trials (Table 1). All trials used different drug regimes. Treatment was adjusted in all trials to response on therapy in previous weeks (different treatment when HCV-RNA was detectable in certain weeks). Rates of SVR are listed in Table 2. For all comparisons between the use of boceprevir or telaprevir and the use of peginterferon alfa and ribavirin (groups 2 and 3) and the use of boceprevir or telaprevir (with or without placebo) only (group 1), there was a significant higher rate of SVR among users of boceprevir or telaprevir ($p<0.001$) (Table 2).

Table 2 - Rates sustained virologic responses (SVRs) and comparisons of SVRs between different study groups

Publication	Study group	Total treatment duration	Sustained Virologic Response (%)	p-value (Group 2 or 3 vs. Group 1) 95% CI (Group B vs. Group A)
Telaprevir not pre-treated	Group 1 (T0 + Placebo12 + PR36)	24 weeks/48 weeks	44	P<0,001
(Jacobson et al., [13])	Group 2 (T8 + Placebo4 + PR12 or PR36)	24 weeks/48 weeks	69	P<0,001
	Group 3 (T12 + PR12 or PR36)	48 weeks	75	
Boceprevir	Group 1 (PR4 + Placebo44)	48 weeks	21	P<0,001
pre- treated	Group 2 (PR4 + B32 (+ Placebo12))	36 weeks/48 weeks	59	P<0,001
(Bacon et al., [14])	Group 3 (PR4 + B44)	48 weeks	69	
Boceprevir not	Group 1 (PR4 +Placebo44)	48 weeks	38	P<0,001
pre-treated (Poordad et al.,	Group 2 (PR4 + B24 (+ Placebo20)	28 weeks / 48 weeks	63	P<0,001
[15])	Group 3 (PR 4 + B44)	48 weeks	66	
Telaprevir not pre-treated	Group A (T12 + PR12)	24 weeks	92	95% CI [-2;11]
(Sherman K et al., [16])	Group B (T12 + PR36) (randomly)**	48 weeks	88	
	Group C (T12 + PR36) (non-randomly)**	48 weeks	64	

Rates of sustained virologic response (SVR) in different study groups following different therapy schemes. Each study group had a different treatment schedule, consisting of telaprevir (T), boceprevir (B), ribavirin (R), peginterferon (P) and/ or a placebo for boceprevir or telaprevir. Duration of treatment varied between 0, 4, 8, 12, 20, 24, 32, 36 or 44 weeks. Statistical analysis was made between the different study groups in the same trial to compare study groups receiving telaprevir or boceprevir (groups 2 and 3) to groups only receiving peginterferon-ribavirin (and placebo) (group 1). Statistical analysis of different durations of telaprevir use (groups A, B, C), was performed between study groups T12 + PR36 (randomly, group B) and T12 +PR12 (group A).

Table 3 - Incidence of adverse events

	Telaprevir not pre-treated (Jacobson, et al.)		Boceprevir not pre-treated (Poordad, et al.)			Boceprevir pre- treated (Bacon, et al.)		
	T12-placebo-PR0-PR12/36	T0-placebo-PR12-PR36	PR4-B44	PR4-placeboPR44	Comparison PR4-B44 vs. PR4-placeboPR44	PR4-B44	PR4-placeboPR44	Comparison PR4-B44 vs. PR4-placeboPR44
% Anemia	37	19	49	29	$p < 0.001$	46	20	$p < 0.001$
% Rash	37	24	-	-		14	5	$p = 0.05$
% Fatigue	57	57	57	60	$p = 0.50$	-	-	
Gastrointestinal disorders								
% Nausea	43	31	43	42	$p = 0.76$	-	-	
% Diarrhea	28	22	-	-		-	-	

Outcomes of rates of adverse events in various studies, subdivided by type of adverse event. Comparisons are made between groups receiving longest duration of telaprevir or boceprevir therapy and groups using only peginterferon-ribavirin (and placebo).

The comparison between the rate of SVR among participants randomly assigned to either 12 weeks extra use of peginterferon alfa and ribavirin (group A, T12 PR12), or to 36 weeks extra use of peginterferon alfa and ribavirin (group B, T12 PR36) in the 'Response-Guided Telepravir Combination Treatment for Hepatitis C Virus Infection' trial, led to no inferiority of only 12 weeks of extra use of peginterferon-ribavirin: the difference in SVR between group B vs. group A was 4% (95% CI [-2;11]), with an inferiority margin of -10,5% or smaller.

Safety

In all studies which included telaprevir or boceprevir regimes compared with peginterferon-ribavirin, we found a higher incidence of most adverse events such as anemia, rash, fatigue and gastrointestinal disorders. In three of the four included studies [13-15] we compared adverse events between study groups receiving the highest dose of telaprevir or boceprevir and the study group receiving peginterferon alfa and ribavirin and/or placebo only. One of the four studies was not appropriate for comparing the safety [16], because duration of telaprevir use was the same in all study groups. Therefore, it was not possible to make a comparison between the adverse events during the use of telaprevir and the use of standard treatment only.

Anemia and rash were the most frequently reported adverse events that led to the discontinuation of telaprevir-based regimens. Anemia was reported as an adverse event in 37% of the telaprevir group vs. 19% of the placebo/peginterferon-ribavirin group. In 37% of the patients in the telaprevir group rash events were reported vs. 24% in the peginterferon-ribavirin group. Gastrointestinal disorders (nausea and diarrhea) were associated with a higher incidence in the telaprevir group (T12-placeboPR0-PR12) compared with the placebo/peginterferon-ribavirin group (T0-placeboPR12-PR36) (43% and 28% vs. 31% and 22%).

Boceprevir use was associated with a significantly higher incidence of anemia ($p<0.001$). Rash was more common in the boceprevir study group (PR4-B44) than in the peginterferon-ribavirin group (PR4-placeboPR44). Use of boceprevir did not lead to a significantly higher incidence of nausea ($p=0.76$).

Both telaprevir and boceprevir study groups were not associated with a higher incidence in fatigue compared to placebo/peginterferon-ribavirin groups.

Discussion

Firstly, our study suggests that the efficacy of combined treatment (peginterferon-ribavirin with protease inhibitor telaprevir or boceprevir) is higher in patients with HCV genotype 1 compared with standard treatment (peginterferon-ribavirin alone). Secondly, our study indicates that combined treatment shows higher rates of adverse events in this patient group relative to standard treatment.

The study suggests that standard treatment of patients with HCV genotype 1 may be adjusted with the addition of a protease inhibitor. However these patients may experience more adverse events, the improved SVR will offer less risk on HCV-related diseases, which are often life-threatening.

The results of our review of randomized controlled phase III studies correspond to the results of the previously cited phase II studies [7-9]. The latter showed a positive correlation of combined treatment (telaprevir combined with peginterferon-ribavirin) with significantly higher response rates. In accordance with phase II studies, our study also shows antiviral effects of boceprevir in patients with HCV genotype 1 [11]. This demonstrates the effect of a protease inhibitor on the inhibition of viral replication, which seems to be beneficial for patients with HCV genotype 1.

Moreover, peginterferon-ribavirin with telaprevir or boceprevir, when compared with peginterferon-ribavirin alone, is associated with a higher incidence of adverse events (anemia and rash). Use of telaprevir is correlated with a higher incidence of gastrointestinal disorders, however it is unclear whether these results are statistically significant, because the individual studies did not provide p-values.

Our results indicate that protease inhibitors are associated with more adverse events. This corresponds to phase II studies, which particularly measured higher incidences of rash in patients who received standard therapy with the addition of telaprevir [7-9]. Due to severe adverse events, such as rash, patients can decide to stop treatment before it is completed. As this happened in phase II studies [7-9], it may be relevant to inform patients about the importance to continue the treatment, despite the adverse events. Furthermore, it should be studied how these adverse events can be limited.

We used the limit 'core clinical journals' to find only articles which examined clinical results of the use of protease inhibitors. After using the search term 'telaprevir or boceprevir' we found 305 articles. By using the limit 'core clinical journals' 273 articles were excluded, so we had 32 articles for our further search.

Because our study population was not limited for sex or race, the results could apply to a wide range of patients.

Limitations

A number of limitations were present in the data. Firstly, none of the studies addressed the use of protease inhibitors in children with HCV genotype 1, which is often acquired through vertical transmission [12]; all participants were 18 years or older.

Therefore our results do not give any evidence of the efficacy and safety of protease inhibitors in these children. Secondly, some data were lacking for accurate analysis of safety.
One of the included publications [16] did not contain sufficient data to determine the safety of telaprevir; the duration of telaprevir was equal in all groups in that study, so no comparison was possible. Thirdly, some of the reviewed publications did not report the statistical significance of certain adverse events. Therefore we cannot assess the validity of these results. Fourthly, we could not review the rates of discontinuation due to use of telaprevir or boceprevir, since these results were not presented in a comparable fashion. Fifthly, although we were able to compare percentages of adverse events between treatment groups with protease inhibitor and control groups, we could not report the statistical significance of the results. A final limitation was that we searched only for publications in English. Therefore, we could have missed important studies published in other languages.

Our results cannot reliably indicate which treatment is the best option, because each study in our review had different treatment schemes. To explore which treatment of HCV genotype 1 patients is most favorable, more studies are needed.

Conclusion

Based on our results, we recommend treatment of HCV genotype 1 with peginterferon-ribavirin combined with protease inhibitor telaprevir or boceprevir.
However, when patients use a protease inhibitor, adverse events should be carefully monitored because of their higher prevalence. Further research into the best treatment scheme and the adverse events is required.

References

1. *World Health Organization. Hepatitis C fact sheet. Fact sheet N°164 June 2011. Available from: http://www.who.int/mediacentre/factsheets/fs164/en/index.html.*
2. *[No authors listed]. Report of a WHO Consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. Global surveillance and control of hepatitis C. J. Viral Hepat. 1999; 1: 35-47.*
3. *Manns MP, McHutchison JG, Gordon SC, Rustgi VK, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet. 2001; 9286: 958-65.*
4. *Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med. 2002; 13: 975-82.*
5. *McHutchison JG, Lawitz EJ, Shiffman ML, et al. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. N Engl J Med. 2009; 6: 580-93.*
6. *Perni RB, Almquist SJ, Byrn RA, et al. Preclinical profile of VX-950, a potent, selective, and orally bioavailable inhibitor of hepatitis C virus NS3-4A serine protease. Antimicrob Agents Chemother. 2006; 3: 899-909.*
7. *McHutchison JG, Everson GT, Gordon SC, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N Engl J Med. 2009; 18: 1827-38.*
8. *Hézode C, Forestier N, Dusheiko G, et al. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med. 2009; 18: 1839-50.*
9. *McHutchison JG, Manns MP, Muir AJ, et al. Telaprevir for previously treated chronic HCV infection. N Engl J Med. 2010; 14: 1292-303.*
10. *Venkatraman S, Bogen SL, Arasappan A, et al. Discovery of (1R,5S)-N-[3-amino-1-(cyclobutylmethyl)-2,3-dioxopropyl]- 3-[2(S)-[[[(1,1-dimethylethyl) amino]carbonyl]amino]-3,3-dimethyl-1-oxobutyl]- 6,6-dimethyl-3-azabicyclo[3.1.0]hexan-2(S)-carboxamide (SCH 503034), a selective, potent, orally bioavailable hepatitis C virus NS3 protease inhibitor: a potential therapeutic agent for the treatment of hepatitis C infection. J Med Chem. 2006; 20: 6074-86.*
11. *Berman K, Kwo PY. Boceprevir, an NS3 protease inhibitor of HCV. Clin Liver Dis. 2009; 3: 429-39.*
12. *Thaler MM, Park CK, Landers DV, et al. Vertical transmission of hepatitis C virus. Lancet. 1991; 8758: 17-8.*
13. *Jacobson IM, McHutchison JG, Dusheiko G, et al. Telaprevir for previously untreated chronic hepatitis C virus infection. N Engl J Med. 2011; 25: 2405-16.*
14. *Bacon BR, Gordon SC, Lawitz E, et al. Boceprevir for previously treated chronic HCV genotype 1 infection. N Engl J Med. 2011; 13: 1207-17.*
15. *Poordad F, McCone J Jr, Bacon BR, et al. Boceprevir for untreated chronic HCV genotype 1 infection. N Engl J Med. 2011; 13: 1195-206.*
16. *Sherman KE, Flamm SL, Afdhal NH, et al. Response-guided telaprevir combination treatment for hepatitis C virus infection. N Engl J Med. 2011; 11: 1014-24.*

Relationship between advanced maternal age and the mode of delivery

A systematic review

Floor Smit[a], Hedwig van Wijk[a], Cornalijn Gouw[a]
Hans Duvekot[b]
[a] Medical student, Erasmus MC University Medical Center Rotterdam, the Netherlands
[b] Supervisor, Department of Gynaecology/Obstetrics, Erasmus MC University Medical Center Rotterdam, the Netherlands
Correspondence: Floor Smit, e-mail: 328264ls@student.eur.nl

Objectives: **The pregnancy rate among older women (women with the age of 35 or above) is increasing and studies have shown that such women undergo more interventions during labor. We therefore addressed the question: what is the relationship between advanced maternal age and the mode of delivery?**
Methods: **We searched PubMed for relevant articles published between 1 January 1990 and 11 January 2012. We excluded systematic reviews and case reports. The main topic of the paper had to be the relationship between mode of delivery and advanced maternal age.**
Results: **We included 10 articles that complied with our inclusion criteria. We found that women of advanced maternal age experienced a higher percentage of cesarean sections and assisted deliveries.**
Conclusion Older women have a higher percentage of intervention during labour. Remarkably, in the group, which consisted of women who gave birth before, the rate of assisted deliveries was higher among the younger women.
Discussion: **The higher rate of caesarean section can be explained by physicians' anxiety for pregnancy outcome in older women.**

Introduction

Because of social, economic and educational developments, it is becoming more common for women of 35 years of age or older to bear children, A maternal age of 35 years and over at the time of delivery has been defined as advanced maternal age[3,1]. Because children of older mothers have more chromosomal anomalies and other congenital abnormalities [2], this could be associated with a higher rate of complications during labor. Moreover, it is likely that obstetricians and other healthcare professional take a different approach towards pregnant women over 35 than towards younger.

This topic has long been a controversial one. In recent decades, many articles have been published on maternal age in relation with mode of delivery. The knowledge on this topic is extensive, and these articles generally reach the conclusion that advanced maternal age can probably be associated with problems during labor[1-13].

The aim of this study was to determine the relationship between advanced maternal age and mode of delivery, specifically cesarean section and assisted vaginal delivery (ventouse and forceps). We also distinguished between multiparous and nulliparous women. Nulliparous means a woman who has never given birth to a viable (live) infant. Multiparous means a woman who has given birth to one or more viable children.
Our review provides gynecologists and obstetricians with a recent overview of the mode of delivery in women of advanced maternal age. The article could help obstetricians decide whether or not to perform a cesarean section in the first instance or not.

We addressed the question, what is the relationship between maternal age and the mode of delivery?

Methods

We used PubMed as a medium to search for articles. We used several MeSH-terms to make our search as specific as possible. We use the following search protocol:

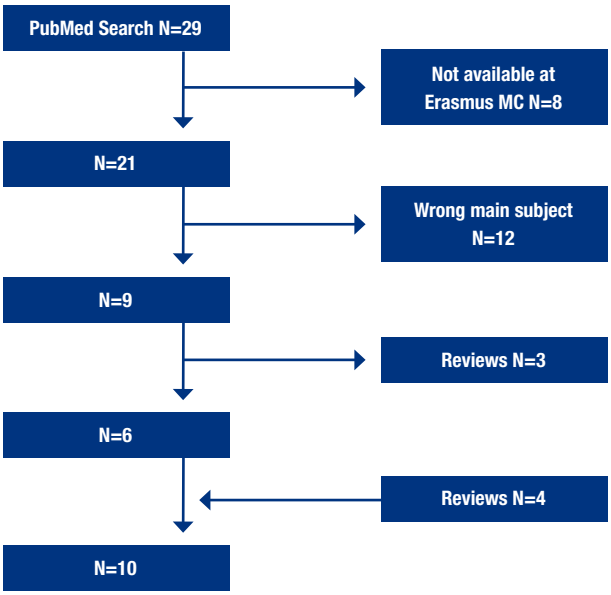
("Maternal Age"[Majr] AND "Pregnancy Outcome"[Mesh]) AND "Cesarean Section"[Mesh] AND ("humans"[MeSH Terms] AND English[lang]) AND ("humans"[MeSH Terms] AND ("women"[MeSH Terms] OR "female"[MeSH Terms]) AND (Clinical Trial[ptyp] OR Practice Guideline[ptyp] OR Randomized Controlled Trial[ptyp] OR Comparative Study[ptyp] OR Controlled Clinical Trial[ptyp] OR "retrospective studies"[MeSH Terms] OR review[ptyp]) AND English[lang] AND "adult"[MeSH Terms] AND ("1990/01/01"[PDAT] : "3000"[PDAT]))

Inclusion and exclusion criteria

The article has to be written in English and be published between January 1, 1990 and January 11, 2012. Articles had to be available in the Erasmus MC library. Following the initial search, we checked the title and the abstracts of the articles for relevance. The main topic had to be the relationship between maternal age and the mode of delivery. Several articles were excluded because they were not sufficiently relevant.

We included only articles based on randomized controlled trials, retrospective studies, comparative studies, case-control studies and controlled clinical trials. Systematic reviews and case reports were exclude. Finally, the articles had to be published in a medical journal.

Schedule 1



We checked the references of the remaining articles to search for more other relevant studies. We used the same inclusion- and exclusion criteria as the articles found on PubMed.

Data analysis

We produced an overview of all the included articles with author, study type, age of the study group (>35 years) and the younger group (<35 years), mode of delivery, parity, indication for cesarean section, p-value and conclusion. We also made an overview of parity. We classified the studies according to whether they differentiated between nulliparous and multiparous or did not. The latter studies were called "both" studies (they included both groups together) then checked the size of the groups (older and younger women)For each classification we then calculated the number of women in the older and /younger groups who had cesarean section/ assisted vaginal deliveries. We divided the number of cesarean sections/assisted vaginal deliveries by the total number in the older/ younger group. The resulting percentage indicated the proportion of cesarean section/assisted vaginal deliveries in the older/younger groups of in all relevant studies.

Results

Inclusion and exclusion

We initially found 29 articles from our PubMed search. We excluded 8 articles because they were not available for the Erasmus MC library. Then, we excluded 12 articles because the main subject was not the relationship between the maternal age and the mode of delivery and. After that, we excluded 3 reviews. The remaining number of articles was 6.

The next step in our search was to check all references, both from the included articles and the excluded reviews, to find additional articles that could also be included in our analysis. This step resulted in 22 articles that were chosen by title. After checking the references in these articles, a total of 4 additional articles were included.

Following these steps, therefore, 10 additional articles were included.

Nulliparous

Table 1 summarizes 7 studies on nulliparous women who delivered by cesarean section. The total number of subjects was 297,842. This was broken down into a study group of 15,463 women and 282,379 younger women. The percentages of these groups delivering by cesarean section were 28.70% and 21.9%, respectively.

Table 1 - Caesarean section in nulliparous woman

Cesarean Section	Younger group (<35 year)	Older group (>35 year)	p-value
Mary Carolan et al.(4)	2321/16920 (21.5%)	1075/9077 (33.8%)	<0.01
VickiLee Edge et al.(5)	285/1597 (18%)	341/857 (40%)	<0.05
Paolo Vercellini et al.(6)	45/148 (30%)	94/148 (64%)	<0.001
Rachana Chibber(7)	8/29 (13.3%)	26/39 (54.2%)	<0.05
S. Ziadeh and A. Yahaya(8)	49/610 (8%)	9/50 (18%)	<0.01
William Gilbert et al.(9)	58252/258.900 (22.5%)	2245/4777 (47%)	<0.05
Oswald Jonas et al.(10)	889/4175 (21.2%)	191/515 (37.1%)	<0.001
New outcome	**61,849/282,379 21.9%**	**3981/15,463 25.70%**	

Table 2 - Assisted vaginal delivery in nulliparous women

Assisted Vaginal Delivery	Younger group (<35 year)	Older group (>35 year)	p-value
Mary Carolan et al.(4)	2559/16,920 (23%)	810/9077 (23.9%)	<0.001
Rachana Chibber(7)	3/29 (4.2%)	4/39 (9.2%)	<0.05
S. Ziadeh and A. Yahaya(8)	38/610 (5.17%)	4/50 (8%)	<0.01
William Gilbert et al.(9)	33.398/258.900 (12.9%)	678/4777 (14.2%)	NS
Oswald Jonas et al.(10)	1210/4175 (28.9%)	180/515 (34.9%)	<0.001
	37,208/280,634 13.30%	**866/5381 16.10%**	

Table 2 summarizes the 5 studies that reported on assisted vaginal deliveries in nulliparous women. The total number of subjects was 286,015. This was divided into group of 5381older women and a group of 280,634 younger women. The percentage of these groups delivering by assisted vaginal delivery was 16,10% and 13.30% respectively.

Multiparous

Table 3 summarizes the 4 articles that reported on multiparous women who delivered by cesarean section. The total number of women in these studies is was 404,501, with 384,628 younger women and 19,873 older women. Of the multiparous young women 17.80% delivered by cesarean section compared to 29.40% multiparous older women.

Table 4 summarizes 3 studies on multiparous women who delivered by assisted vaginal delivery. The total number of subjects was 404,143. This was broken down into a group of 384,449 younger women and a group of 19,694 older women of which 14,60% and 6,3% respectively, delivered by assisted vaginal delivery.

Both

Table 5 summarizes 3 articles which did not distinguish between nulliparous and multiparous women who delivered by Caesarean section. The total number of subjects in these studies together was 109,640. This included a group of 109,287 younger women and a group of 353 older women. The percentages of these groups delivering by cesarean section were 22,90% and 37,70%, respectively.

Finally, Table 6 summarizes 3 studies on assisted vaginal delivery that did not distinguish between nulliparous and multiparous women. The total number of subjects was 696. This included a group of 496 younger women and a group of 277 older women. The percentages of these groups delivering by assisted vaginal delivery were 3.60% and 7.60%, respectively.

Table 3 - Cesarean section in multiparous women

Cesarean Section	Younger group (<35 year)		Older group (>35 year)		p-value
Paolo Vercellini et al.(6)	21/179 (12%)		77/179 (43%)		<0.001
Rachana Chibber(7)	3/20 (5.1%)		9/21 (18.4%)		<0.05
S. Ziadeh and A. Yahaya(8)	48/794 (6%)		58/418 (14%)		<0.01
William Gilbert et al.(9)	68285/383,635 (17.8%)		5699/19.255 (29,6%)		<0.05
New outcome	**68,357/384,628**	**17.80%**	**5843/19,873**	**29.40%**	

Table 4 - Assisted vaginal delivery in multiparous women

Assisted Vaginal Delivery	Younger group (<35 year)		Older group (>35 year)		p-value
Rachana Chibber(7)	1/20 (2.1%)		1/21 (3.2%)		<0.05
S. Ziadeh and A. Yahaya(8)	38/794 (4.75%)		26/418 (6.2%)		<0.01
William Gilbert et al.(9)	56.009/383.635 (4.6%)		1213/19.255 (6.3%)		<0.05
	56,048/384,449	**14.60%**	**1239/19,694**	**6.30%**	

Table 5 - Cesarean section, no destinction between nulliparous an multiparous women

Cesarean Section	Younger group (<35 year)		Older group (>35 year)		p-value
Leonie K. Callaway et al.(11)	25,010/108,818 (23%)		38/76 (49%)		<0.05
Michael F.E. Diejomaoh et al.(12)	26/160 (16.3%)		52/168 (31.1%)		<0.0027
Mordechai Dulitzki et al.(13)	13/309 (4.2)		43/109 (39.4%)		<0.001
	25,049/109,287	**22.90%**	**133/353**	**37.70%**	

Table 6 - Assisted vaginal delivery, no distinction between nulliparous and multiparous women

Assisted Vaginal Delivery	Younger group (<35 year)		Older group (>35 year)		p-value
Leonie K. Callaway et al.(11)	unknown		3 (3.8%)		NS
Michael F.E. Diejomaoh et al.(12)	7/160 (4.4%)		12 (7.1%)		NS
Mordechai Dulitzki et al.(13)	10/309 (3.2%)		9 (8.3%)		<0.001
	17/469	**3.6%**	**21/277**	**7.60%**	

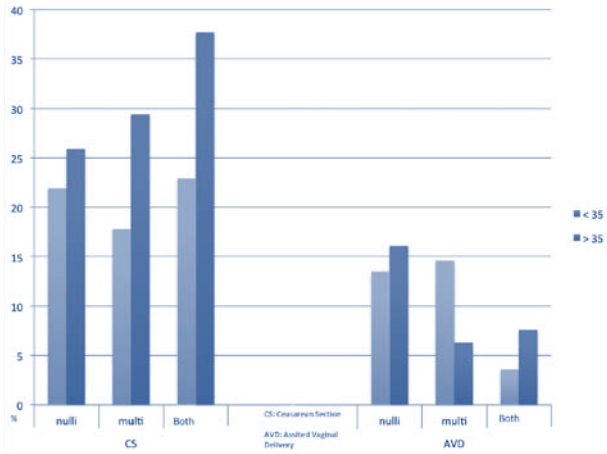


Figure 1 - Histogram comparing cesarean section and assisted vaginal delivery

Discussion

In our review, we wanted to assess the relationship between advanced maternal age and the mode of delivery. After searching PubMed and references for relevant articles, we included 10 studies in our review. We compared the groups of women as described above.

The results of our review and analysis suggest a relationship between maternal age and the mode of delivery. In almost every situation we found that older woman have a higher risk of intervention during labor. The biggest difference between older and younger women was found in the studies on cesarean deliveries that did not distinguish between nulliparous and multiparous women. Unexpectedly, the rate of assisted vaginal deliveries was twice as high in the younger multiparous group than in the older multiparous group.

The specific reasons for the increased incidence of cesarean delivery in older women are unclear. Older women are at higher risk for complications of pregnancy that are associated with cesarean section, and they have a greater likelihood of poor medical status [4]. But maternal age alone may also influence a physician's decision regarding method of delivery. In addition, physicians' concerns about pregnancy outcomes in older women and couples' demands for perfect pregnancy outcomes could also affect the choice for mode of delivery. To find out the specific reasons for the increased incidence more research is required.

One reason for physicians to choose a cesarean section delivery in older women is their decreasing strength in the uterus and reduced elasticity of the pelvic joints [11]. Moreover, more fetal stress has been shown in women older than 35 years [2]. Although the overall risk of complications during childbirth is clearly higher in older women, we hypothesize that the higher rate of cesarean sections in women older than 35 years is not only a consequence of a decreased overall condition and other physical causes, but that emotional and psychological reasons also play a role.

First of all older mothers are probably more anxious about complications when giving birth, and physicians therefore tend to be more accommodating towards those women by performing a cesarean section. Not only could they be anxious because they are older and have more knowledge about childbirth risks, but the couple could also have infertility problems. This could be one reason why older women have less confidence in a positive outcome [7].

Furthermore obstetricians tend to be more concerned about older women giving birth because they could have weaker muscles and would perhaps have to exert even more effort to deliver a baby. Obstetricians fear negative pregnancy outcomes in older women [11].

A final point of discussion concerns results from the studies on cesarean section that did not distinguish between nulliparous and multiparous women. Because it is unknown how many nulliparous and multiparous women were included in this study population, it is more difficult to draw conclusions. Therefore, we conclude that the group of studies that did distinguish between these groups of women should be given the greatest clinical relevance.

Limitations

One limitation is the small number of articles in our meta-analysis. However, the total number of subjects is large and we believe that this sample size is large enough to draw meaningful conclusions. A second possible limitation is that the largest study by far was performed in the United States of America, which could lead to an overly limited variety in the study group. However the demographic variety in the United States of America is so large that it would probably not affect our study results. Another possible limitation is the wide deviation between the sizes of the groups of older and younger women in the various studies. Specifically, the Gilbert study [9] involved such a large number of subjects that our results could have been dominated by the outcome of this single study. However, the fact that the most of the other studies also found an association between advanced maternal age and a higher rate of cesarean sections indicates that our results are reliable.

Table 7 - Overview included article

VickiLee Edge et al.	Prospective cohort study	>35 N = 857	20-29 N = 1597	Caesarean section	nulliparous	Physician's fear for pregnancy outcomes in older women.	p < 0.05	Women of 35 or older are more likely than those in their 20s to have caesarean deliveries.
Paolo Vercellini et al.	Case-control study	>40 N = 327	20-30 N = 327	Caesarean section	nulliparous, multiparous and both	An extremely conservative approach rather than an increased incidence of complications of labor.	Nulliparous; p < 0.001; multiparous; p < 0.001	Our findings confirm the elevated caesarean section rate in older gravidas. However, it has not been definitly proven that an elective ceasarean section merely on the basis of maternal age is adequeate obstetric policy.
Leonie K. Callaway et al.	Retrospective cohort study	≥ 45 N = 76	20 - 29 N = 108818	Caesarean section and assisted vaginal delivery	both	Obstetrician and patient preference, the impact of ageing on myometrial efficiency and elasticity of pelvic joints, and age related confounders including fetal stress.	CS; p < 0,05; AVD; p=NS	There was a significant difference in the rate of caesarean delivery between women of very advanced maternal age and younger patients.
Rachana Chibber	Retrospective cohort study	≥ 50 N = 49	20-29 N = 60	Caesarean section and assisted vaginal delivery	nulliparous and multiparous	Ante-partum complications, couples desire and demand for perfect pregnancy outcomes, provider's anxiety.	Nulliparous; p < 0.05; multiparous; p < 0,05	Pregnancy and successful delivery may be expected in healthy women in their late 50s. Women choosing motherhood in this group may expect a high rate of caesarean delivery.
Michael F.E. Diejomaoh et al.	Retrospective cohort study	40 - 47 N = 168 ≥ 40 N = 468	25 - 30 N = 160 20 – 29 N = 309	Caesarean section and assisted vaginal delivery Caesarean section and assisted vaginal delivery	both nulliparous and multiparous	Medical disorders, obstetric complications and fetal indications. And obstetricians may have a lowered threshold for caesarean section in women of advanced age.	CS; p < 0,05; AVD; p=NS	Advanced maternal age of 40 yearts and over was not associated with adverse maternal en perinatal outcome, although the incidence of caesarean section was significantly increased in these women.
S. Ziadeh and A. Yahaya	Case-control study	≥ 44 N = 109	20 – 29 N = 309	Caesarean section and assisted vaginal delivery	both	Maternal age alone may influence a physician's decision regarding method of delivery.	Nulliparous CS; p < 0,01; multiparous CS; p < 0,01; nulliparous AVD; p < 0,01; multiparous AVD; p < 0,01	Nulliparous women age 40 or over have a higher risk of operative delivery than do younger nulliparous women.
Mordechai Dulitzki et al	Case-control study	≥ 40 N = 24032	20-29 N = 642525	Caesarean section and assisted vaginal delivery	nulliparous and multiparous	The specific reasons for increased caesarean delivery incidence in older women has not yet been determined.	CS; p < 0,001; AVD; p < 0,001	Maternal age of at least 44 years is associated with medical complications in pregnancy and more interventions during labor.
Willliam Gilbert et al.	Retrospective study					Antepartum or intrapartum complications, couples' demands for perfect pregnancy outcomes, provider's anxiety.	Nulliparous CS; p < 0.05; multiparous CS; p < 0,05; nulliparous AVD; p=NS; multiparous AVD; p < 0,05	This study shows that first-time mothers who are giving birth at age 40 or older are at high risk for some form of operative delivery.
Oswald Jonas et al.	Retrospective cohort study	≥ 35 N = 515	20-29 N = 4175	Caesarean section and assisted vaginal delivery	nulliparous	No indications found	CS; p < 0,001; AVD; p < 0,001	For primigravid women the risk of medical, obstetric and labour complications was greater among women aged 35 years or older, than for 20-29 year olds. The caesarean section rate among the older primigravid women was almost twice that of their younger counterparts.

References

1. *Benzies K, Tough S et al. Factors influencing women's decisions about timing of motherhood. J Obstet Gynecol Neonatal Nurs. 2006 ; 5: 625-33.*
2. *Joseph KS, Allen AC et al. The perinatal effects of delayed childbearing. Obstet Gynecol. 2005; 6: 1410-8.*
3. *Carolan M, Davey MA et al. Older maternal age and intervention in labor: a population-based study comparing older and younger first-time mothers in Victoria, Australia. Birth. 2011; 1: 24-9.*
4. *Bayrampour H, Heaman M. Advanced maternal age and the risk of cesarean birth: a systematic review. Birth. 2010; 3: 219-26.*
5. *Edge V, Laros RK, Jr. Pregnancy outcome in nulliparous women aged 35 or older. Am J Obstet Gynecol. 1993; 6 Pt 1: 1881-4; discussion 4-5.*
6. *Vercellini P, Zuliani G et al. Pregnancy at forty and over: a case-control study. Eur J Obstet Gynecol Reprod Biol. 1993; 3: 191-5.*
7. *Chibber R. Child-bearing beyond age 50: pregnancy outcome in 59 cases "a concern?". Arch Gynecol Obstet. 2005; 3: 189-94.*
8. *Ziadeh SM. Maternal and perinatal outcome in nulliparous women aged 35 and older. Gynecol Obstet Invest. 2002; 1: 6-10.*
9. *Gilbert WM, Nesbitt TS et al. Childbearing beyond age 40: pregnancy outcome in 24,032 cases. Obstet Gynecol. 1999; 1: 9-14.*
10. *Jonas O, Chan A et al. Pregnancy outcomes in primigravid women aged 35 years and over in South Australia, 1986-1988. Med J Aust. 1991 18; 4: 246-9.*
11. *Callaway LK, Lust K et al. Pregnancy outcomes in women of very advanced maternal age. Aust N Z J Obstet Gynaecol. 2005; 1: 12-6.*
12. *Diejomaoh MF, Al-Shamali IA et al. The reproductive performance of women at 40 years and over. Eur J Obstet Gynecol Reprod Biol. 2006 1; 1: 33-8.*
13. *Dulitzki M, Soriano D et al. Effect of very advanced maternal age on pregnancy outcome and rate of cesarean delivery. Obstet Gynecol. 1998; 6: 935-9.*

Adult versus Pediatric Renal Allograft in Children: a comparison based on GFR and graft survival

A systematic review

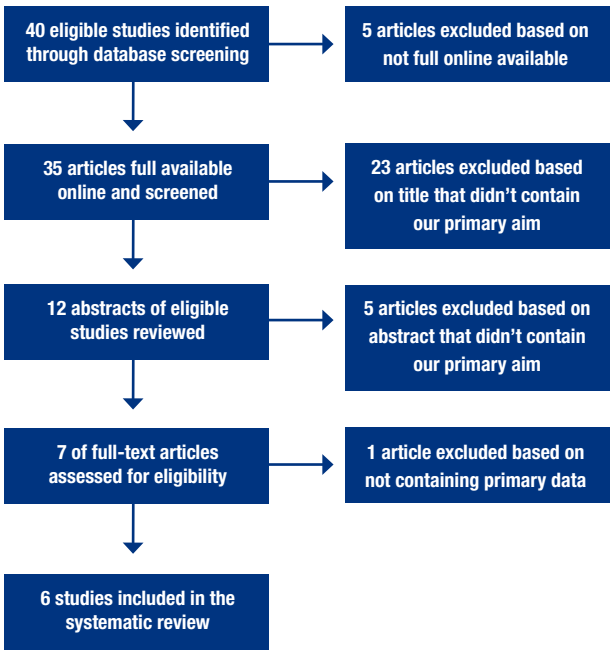
Meike van Amelsfort[a], Julia Kobben[a], Sophia Kronig[a], Rozemarijn van der Mooren[a], Stella Nieuwenburg[a], Karlien Cransberg[b]
[a]Medical student, Erasmus MC University Medical Center Rotterdam, the Netherlands
[b]Supervisor, Department of Pediatric Nephrology, Erasmus MC/Sophia Children's Hospital, Rotterdam, the Netherlands
Correspondence: Sophia Kronig, e-mail: 340458sk@student.eur.nl

Objective: **Renal transplantation has become the treatment of choice in children with end-stage renal disease. In this review we evaluated published data on the difference in renal function and graft survival between pediatric and adult donor grafts in pediatric kidney transplantation.**
Methods: **A systematic review was performed using the PubMed database. We retrieved all published, English-language, original articles which had as a primary outcome the comparison of renal function and graft survival in pediatric and adult kidney grafts transplanted into children. The full text had to be available online.**
Results: **The search yielded 6 eligible articles. No significant difference was found in graft survival between adult and pediatric cadaveric donor grafts, except in the study by Dubourg et al. They reported a superior graft survival rate with living related donor grafts (LRD) compared to cadaveric grafts, both adult and pediatric. Adult donor grafts maintained a constant absolute glomerular filtration rate (GFR), leading to a significant decrease in relative GFR as the children grew. Pediatric donor grafts showed a significant increase in absolute GFR during the follow up, leading to a stable relative GFR.**
Conclusions: **Based on renal function, we conclude that children benefit more from a pediatric kidney graft than from an adult kidney graft.**

Introduction

Over the last 30 years, renal transplantation has become the treatment of choice in children with end-stage renal disease. Even though medical technology has developed substantially in recent decades, there are still obstacles to successful transplantation caused by both immunologic and non-immunologic factors [1,2]. In this review we focused on one non-immunologic factor: the effect of graft size on the outcome of graft survival and graft function in pediatric recipients. Pediatric kidney grafts could have specific benefits; a kidney can grow with the child recipient and the glomerular filtration rate (GFR) can increase over the years, leading to a stable relative GFR. Relative GFR refers to GFR corrected for body surface area. Bearing in mind the lower graft survival after dialysis, pre-emptive transplantation from a living donor is preferred [3]. Organs from a living donor always originate from an adult donor, since children may not donate by law. We searched the literature to address the following research question: based on GFR and renal function, which allograft, pediatric or adult, is best to transplant into children?

Figure 1 - Flow diagram of data selection



Methods

Search strategy and data selection

Between 9 and 16 January 2012, we searched for English-language articles in the PubMed electronic database. The search was conducted by 5 reviewers, and a senior reviewer resolved disagreements. We did not use exclusion criteria for the data. We searched using various combinations of keywords including kidney transplantation, pediatric recipient, adult donor, renal function and graft survival. In our search strategy we used the following MeSH terms: "kidney transplantation"[Mesh] AND "child"[Mesh] AND "adult "[Mesh] AND ("Kidney Function Tests"[MeSH] OR "GFR" [TW]) AND ("Graft Survival/ physiology" [MeSH] OR "growth"[TW]) AND ("humans"[MeSHTerms] AND English [lang]).

Articles had to meet the following inclusion criteria: 1) the primary outcome is a comparison between pediatric and adult kidney grafts transplanted into children, based on renal function and graft survival; 2) it is an original study; 3) the full text is available online; 4) the article is published in English.

The reference lists of the identified articles were also checked for additional studies missed by the PubMed search, but no other eligible articles were found.

Our PubMed search produced 40 eligible publications, of which 35 were available (full text) online. Titles were first reviewed to determine if a study met our inclusion criteria, resulting in 12 studies. Next, the abstracts of the 12 eligible articles were read; 5 of these studies, which did not contain a comparison between adult and pediatric kidney grafts transplanted into children, were then excluded. After reading the full studies, one more article was excluded because it did not contain new data based on the study population. As a result, we were left with 6 relevant articles that met all of our criteria and which were selected for our systematic review (Figure 1). We have summarized the methods used in these studies in Table 1.

Results

Follow up

The follow-up time in the 6 studies differed (Table 1). Feltran et al. used at least one year follow up, while Pape et al. had a mean observation time of 5.9 years with a standard deviation of 4 [4,5]. Pape et al. used a follow up time of 7 years and the study of Dubourg et al. had a minimum follow-up of 4 years and a maximum of 8 years [6,7]. The study of Berg et al. followed their patients for 0.9 to 13.8 years, with a median follow up of 5 years and Gellert et al. used 4 years [8,9].

As part of the follow up, all studies measured the creatinine levels and calculated the absolute and/or relative GFR (adjusted to body surface area – BSA).

Table 1 - Methods of eligible studies

Article [ref]	Single/ Multi center	Type of study	Study population size (N children (age))	Period of inclusion	Primary aim; comparison donors in:	Follow-up time (years)
L. de Santis Feltran et al. 2010 [4]	Single center	Observational, prospective study	N=68 (3-18): 36= PedCD 32=LRD	2006-2008	GFR	1
L. Pape et al. 2006 [5]	Single center	Observational, retrospective study	N=99 (<10): 63= AdCD 39= PedCD	1990-2005	GFR, GS	MOT= 5.9, SD =4.0
L. Pape et al. 2004 [6]	Multi center	Retrospective cohort study	N=15 (1-9): 9=AdCD 6=PedCD	-	GFR, GS	7
L. Dubourg et al. 2002 [7]	Multi center	Observational, retrospective study	N= 134(0.4-18): 13= AdCD 59=PedCD 62=LRD	1982-1999	GFR, GS	4-8
U. Berg et al. 1997 [8]	Single center	Observational, prospective study	N=85 (0.4-20.5) 90 Ktx 68=LRD 22=CD	-	GFR	MOT=5, (0.9-13.8)
S. Gellert et al. 1996 [9]	Single center	Retrospective and Prospective study	N= 105 114 Ktx 52= PedCD 62=Adult donor	-	GFR, GS	MOT=4

AdCD: Adult Cadaveric Donor; GFR: Glomerular Filtration Rate (graft survival); GS: Graft Survival; KTx: Kidney Transplantation; LRD: Living Related Donor; MOT: Mean Observation Time; PedCD: Pediatric Cadaveric Donor

Table 2 - Relative GFR of pediatric and adult donors according to time after transplantation

Article	3 months	6 months	1 year	3 years	4 years	8 years
Feltran [4]	76±22 vs. 100±20 *	88±31 vs. 97±29	102±32 vs. 99±27	-	-	-
Pape [5]	-	-	67±21 vs. 64±18	69±23 vs. 56±16 *	68±28 vs. 53±17 *	-
Dubourg [7]	-	65±23 vs. 71±25	-	-	70±25 vs. 52±19 *	57±19 vs. 45±19 *
Gellert [9]	62±20 vs. 61±21	-	-	-	60±21 vs. 54±20	-

GFR = ml/min/1.73m²; (*) = significant difference;($p<0.05$); (-) = no data

Patient selection

The single-center study of Feltran et al. took place from 2006 until 2008, during which 91 kidney transplantations were performed. Of these patients, 31 were excluded because the child recipients received an adult cadaveric donor (AdCD) or follow up was not performed at the center [4]. Pape et al. selected their study population from kidney transplantations performed between 1990 and 2005. All children who received their first cadaveric kidney allograft were included [5]. Pape et al. used the Eurotransplant registry to select patients [6]. From 1982 until 1999, 294 kidney transplantations were performed in the multicenter study of Dubourg et al. They included 134 patients in their study [7]. Gellert et al. included all pediatric kidney transplantations at a single center [9]. The study of Berg et al. contained no information on the method of patient selection [8].

Immunosuppression

The same main combination of immunosuppressive drugs was used in 4 studies: prednisone, cyclosporine and azathioprine [6,7,8,9]. Feltran et al. used tacrolimus, prednisone and azathioprine, associated with basiliximab induction [4]. Pape et al. used prednisone and cyclosporine A as immunosuppressive drugs, after 1998 in combination with mycophenolate mofetil [5].

Graft survival

Four studies [5,6,7,9] made a comparison of graft survival rates between adult donor grafts and pediatric donor grafts in children. None of the studies found a significant difference in graft survival between adult and pediatric cadaveric donors. Dubourg et al. reported a superior graft survival rate with living related donors (LRD) compared to cadaveric adult or pediatric donors ($p<0.01$). The estimated survival rates derived from the chart were 90% (LRD), 62% (cadaveric adult donors) and 73% (pediatric donors) [7]. The main reasons for graft loss were comparable in the various studies and included chronic allograft nephropathy, severe infection (e.g. CMV reactivation) and acute rejection [9].

Renal function: Glomerular Filtration Rate (Table 2)

All 6 studies determined GFR, but only 4 studies clarified their findings with figures, as shown in Table 2. For the assessment of GFR, 2 out of 4 studies [4, 5, 7] used a calculated relative GFR [10]. Dubourg et al. used inulin clearance, and Gellert et al. used a method with isotopes to measure GFR [9]. Due to this variety of methods we were unable to compare the values themselves; only the trends within the studies were compared.

Two studies compared the absolute GFR and relative GFR (adjusted to BSA) of adult and pediatric donor grafts transplanted into children. Feltran et al. reported that the relative GFR of pediatric donor grafts compared to adult donor grafts is significantly lower at three months after transplantation (76±22 vs. 100±20) [4], in contrast to Gellert et al., who found similar GFR values [9]. During follow up, this difference in GFR converges to a similar GFR between donor grafts. The relative GFR of pediatric donor grafts ultimately became significantly higher compared to adult donor grafts [7].

Feltran et al. reported no significant difference in GFR between donor grafts starting from the sixth month of follow up (88±31 vs. 97±29) [4]. Pape et al. showed that the relative GFR in pediatric donor grafts became significantly higher after three years of follow up (69±23 vs. 56±16) [5]. Dubourg reached the same conclusion, but after four years of follow up (70±25 vs. 52±19) [7].

Three studies [4,7,8] concluded that adult donor grafts keep a constant absolute GFR, leading to a significant decrease in relative GFR proportional to the growth of the child ($p<0.0001$). Pediatric donor grafts showed a significant increase in absolute GFR during the follow up, leading to a stable relative GFR ($p<0.01$) [4,7,8]. Dubourg et al. reported that at 8 years after transplantation, relative GFR in both adult and pediatric kidneys showed a slow decrease, but relative GFR in pediatric donor grafts was still significantly higher than the adult donor grafts (57±19 vs. 45±19) ($p<0.05$) [7].

One study [9] concluded that GFR of pediatric donor grafts remained unchanged until four years after transplantation (60±21), while GFR of adult donor grafts showed a slight decrease (54±20), but this difference was not significant.

Discussion

This systematic review shows that children benefit more from a pediatric kidney graft than from an adult kidney graft, based on GFR and renal function.

Taking the studies together, the data suggest that during the 1st year after transplantation, relative GFR increases in the pediatric donors and remains stable in the adult donors, while in following years the function of the pediatric grafts remains stable, while that of the adult donors gradually declines. No significant difference in graft survival between adult and pediatric kidney grafts transplanted into children was found.

However, there was a significant difference in renal function between the grafts after transplantation. Adult donor grafts maintained a constant absolute GFR, leading to a significant decrease in relative GFR proportional to the growth of the child. Pediatric donor grafts showed a significant increase in absolute GFR during the follow up, leading to a stable relative GFR.

Limitations

Based on our search criteria and full text availability, only 6 articles were eligible for our systematic review. Our primary search yielded 40 eligible articles, of which we excluded 5 articles because they were not available online. However, we did not verify if these articles met our other inclusion criteria; therefore we may have missed some relevant articles.

The studies did not use the same immunosuppressive scheme, primarily because the protocols were unique to specific medical centers and specific time periods. However, within the individual studies, the main combinations of immunosuppressive drugs were the same.

Furthermore, we were surprised that 3 out of 4 studies did not report a significant difference in graft survival between adult and pediatric donors. The relative GFR in pediatric donor grafts is sig-

nificantly higher than in adult donor grafts, so one would logically expect graft survival to increase together with renal function. This contradiction is probably due to the fact that patients with graft loss were excluded when calculating GFR. The authors did not give any explanations for these results.

We did not include the study of Pape et al. [6] in the GFR section of the results because the written text contradicted a figure in the publication. Pape et al. stated that pediatric recipients who received a pediatric donor kidney had a lower calculated GFR 1,2,3,4 and 7 years after transplantation. However, the figure they referred to showed that pediatric recipients with an adult donor kidney were the ones with a significantly lower calculated GFR in the years after transplantation [6]. Therefore we could not include these results in this section, although we did include the data of Pape et al. in the rest of our review.

We also did not use the GFR data from two studies [6,8] in Table 2 because these authors did not report specific values of GFR; they presented their results only in graphs, from which accurate data could not be derived.

Gellert et al. [9] made no distinction between adult living and adult cadaveric donors; they simply combined them in the term "adult donor". This could have affected their results in the comparison of renal function between donors, as it is known that living donors have a better outcome than cadaveric donors.

Conclusion

Based on our systematic review we conclude that pediatric recipients benefit from receiving a pediatric donor graft, since the relative GFR of pediatric grafts increases over time, while the relative GFR of adult donor grafts decreases. This could be partially explained by the loss of functioning nephrons in adult grafts when confronted with the lower blood pressure of the young recipients.

Kidney transplantation with an adult living donor has certain benefits: it is an elective procedure, the ischemic time is lower and more donor options are possibly available. Nevertheless, our conclusion is that children can benefit more from a pediatric kidney graft than from an adult kidney graft. Additional studies are needed to make an evidence-based conclusion on this issue.

Furthermore, more research on the effects of renal pediatric allografts in adult recipients is needed to make a more definitive conclusion about giving all available renal pediatric allografts to child recipients. Additionally, the effects of morbidity and mortality of dialysis while awaiting a kidney from a deceased pediatric donor should be compared with the possible advantages of early transplantation if a living donor is available or in case of earlier availability of a deceased adult donor.

References

1. *Chertow, G.M., Brenner, B.M., Mackenzie, H.S., et al. Non-immunologic predictors of chronic renal allograft failure: Data from the United Network of Organ Sharing. Kidney Int Suppl. 1995; 52: 48-51.*
2. *Kasiske, B.L., Snyder, J.J., Gilbertson, D. Inadequate donor size in cadaver kidney transplantation. J Am Soc Nephrol. 2002; 8: 2152-2159.*
3. *Cransberg, K., Smits, J.M., Offner, G., et al. Kidney transplantation without prior dialysis in children: the Eurotransplant experience. Am J Transplant. 2006; 8: 1858-1864.*
4. *de Santis Feltran, L., Nogueira, P.C.K., Silva, F.A.B., et al. A one year prospective comparison of kidney growth and function in children recipients of grafts from children and adults. Transplantation. 2010; 90: 777-781.*
5. *Pape, L., Hoppe, J., Becker, T., et al. Superior long-term graft function and better growth of grafts in children receiving kidneys from paediatric compared with adult donors. Nephrol dial Transplant. 2006; 21: 2596-2600.*
6. *Pape, L., Offner, G., Ehrich, J.H.H., et al. Renal allograft function in matched pediatric and adult recipient pairs of the same donor. Transplantation. 2004; 77: 1191-1194.*
7. *Dubourg, L., Cochat, P., Haj-Aïssa, A., et al. Better long-term functional adaptation to the child's size with pediatric compared to adult kidney donors. Kidney International. 2002; 62: 1454-1460.*
8. *Berg, U.B., Bohlin, A., Tydén ,G. Influence of donor and recipient ages and sex on graft function after pediatric renal transplantation. Transplantation. 1997; 10: 1424-1428.*
9. *Gellert, S., Devaux, S., Schönberger, B., et al. Donor age and graft function. Pediatr Nephrol. 1996; 10: 716-719.*
10. *Schwartz, G.J., Brion, L.P., Spitzer, A. The use of plasma creatinine concentration for estimating glomerular filtration in infants, children and adolescents. Ped Clin N Am. 1987; 34: 318-322.*
11. *Dubourg, L., Hadj-Aïssa, A., Parchoux, B., et al. Role of the donor in post-transplant renal function. Nephrol Dial Transplant. 1998; 6: 1494-1498.*

Does antioxidant supplementation improve symptoms in schizophrenia patients *A systematic review*

Alis Heshmatollah[a], Madelon M.E. de Jong[a], Hemmo A. Drexhage[b]
[a] Medical student, Erasmus University Medical Center Rotterdam, the Netherlands
[b] Supervisor, Department of Immunology, Erasmus University Medical Center Rotterdam
Correspondence: Alis Heshmatollah, email: 339395ah@student.eur.nl

Objective: **The aim of this review was to determine whether the use of antioxidant supplementation, in addition to antipsychotics, improves symptoms in patients with schizophrenia.**
Methods: **Included articles were found by searching with appropriate search terms in the PubMed database. Studies on the benefits of antioxidant treatment in schizophrenia using psychiatric scales were included. Studies on prevention or treatment of side effects were excluded.**
Results: **Four studies met the selection criteria and were included. SAPS (positive symptom scale) was significantly improved in the treatment group vs. placebo group in two of three studies (mean 3.9 p=0.026, mean 6.5 p<0.05 vs. mean -0.5 p>0.05). SANS (negative symptom scale) was significantly improved in the treatment group in one of three studies (mean 3.3 p=0.08, MD -1.5 p>0,05, mean 1.8 p<0.05). In one of two studies BPRS (brief psychiatric scale) was also significantly improved in the treatment group (mean 9.66 p<0.01 vs. mean 2.9 p=0.058).**
Conclusions: **Our review suggests that antioxidant supplementation may benefit symptomatic schizophrenia patients, especially those suffering from positive symptoms.**

Introduction

The pathophysiology of schizophrenia is very complex, which makes it difficult to find a suitable treatment for this disorder [1]. Currently, patients are most often treated using antipsychotics such as haloperidol [2]. However, in 50% of the cases, these antipsychotics do not offer enough relief, and in 30% of the cases the patients still present with disturbed behavior [3]. If an improved therapy that reduces these symptoms was available, this could substantially improve the patients' quality of life.

Oxidative stress in the brain of a schizophrenia patient plays a role in the development of the disease. The stress results from the presence of chemical radicals. A major source of radicals in the human body is dioxygen (O_2). The radicals originating from O_2 are called reactive oxygen species (ROS) [4]. A number of studies have shown that excess free radical formation occurs in the brains of patients with schizophrenia or depression [5,6]. This has led to the examination of drugs which could possibly interfere with this process. A number of antioxidants have been selected as possible treatments. In this review we included Gingko biloba extract, N-acetyl cysteine and vitamin C (ascorbic acid). These antioxidants were used most often in the studies we reviewed.

Gingko biloba extract is derived from and named after an ancient Chinese tree, which was held sacred for its health promoting properties [7]. Today, the plant extract called 'EGb-761' is used widely in cerebrovascular insufficiency and cognitive and functional symptoms in dementia [8]. Although its underlying mechanism is not completely understood, numerous studies have indicated an antioxidant effect [9].

One study showed that administering EGb-761 to immune-suppressed rats increased the cellular immune response close to control group values [10], while another study showed that EGb-761 administration effectively reversed age-related decrease of immune function in old mice [11].

Ascorbic acid functions as a co-enzyme in more than 800 biochemical reactions in the human body [12] and is more abundantly present in the brain than other organs. Its antioxidant effect has been long known, and sufficient daily intake of this vitamin is recommended [13].

N-acetyl cysteine is a precursor of the antioxidant glutathione [14]. Levels of this antioxidant were found to be lower in the cerebrospinal fluid of 27% of drug-naive patients suffering from schizophrenia [15]. It is hypothesized that this reduction is caused by polymorphisms in the genes for glutamate cysteine ligase modifier subunit [16] and the catalytic subunit for glutamate cysteine ligase [17], which both participate in glutathione synthesis. Cysteine is the rate-limiting precursor of glutathione. Although oral supplementation of pure cysteine is not efficiently bio-available [18], N-acetyl cysteine is efficiently bio available and can also increase plasma glutathione [19].

The aim of this review was to determine whether the use of antioxidant supplementation, along with antipsychotics, reduces symptoms in patients with schizophrenia. We only included studies that used psychiatric scales as a benchmark. These measurement methods are used in most studies, since they are easily applicable in the clinic.

Methods

Search method

PubMed was used as the database to find suitable articles. The search was carried out on 21 January 2012. We limited the search to 'randomized controlled trial', since this is the least biased type of study.

The following query was used: "Schizophrenia"[MeSH] AND "Antioxidants" [MeSH] AND Randomized Controlled Trial [ptyp].

Inclusion criteria were: 1) the evaluation of the benefits of antioxidant treatment for schizophrenia patients, 2) the use of valid psychiatric scales such as Scale for Assessment of Positive Symptoms (SAPS) or Brief Psychiatric Rating Scale (BPRS) and 3) the article was written in English, Dutch, German or French.

In our review we focused on the primary treatment of schizophrenia with antioxidants; we therefore excluded articles 1) on prevention and 2) on reducing side effects of antipsychotics.

Symptom assessment scales

Articles that used valid psychiatric scales [20] were included. In three of the articles [24-26] the Positive and Negative Syndrome Scale (PANSS) was used. In two of these articles [24,25], PANSS was split into Scale for Assessment of Positive Symptoms (SAPS) and Scale for Assessment of Negative Symptoms (SANS). The Brief Psychiatric Rating Scale (BPRS) [24, 27] and CGI-I/S were also used [26].

SAPS is designed to assess positive symptoms, principally those that occur in schizophrenia. These symptoms, such as delusions, hallucinations, hostility and hyperactivity, are evaluated in a clinical interview lasting approximately 45 minutes [21].
SANS is used to evaluate the negative symptoms, like emotional withdrawal, poor rapport and apathetic social withdrawal [21].
Psychiatric symptoms can also be scored with the BPRS. As many as 24 symptoms such as anxiety, guilt, tension and motor retardation are graded with this scale [22].

Finally, the Clinical Global Impression scale (CGI) measures symptom severity, treatment response and the efficacy of therapies in treatment studies. It is divided into two subscales: CGI-severity and CGI-improvement. The CGI-severity scale (CGI-S) is a 7-point scale that requires a clinician to rate the severity of the illness of the patient at the time of assessment. This is based on the clinician's past experience with patients suffering from the same mental disorder. The CGI improvement scale (CGI-I) is a 7-point scale that requires the clinician to assess how much the illness of the patient has improved or worsened compared to baseline [23].

To understand the mechanism of antioxidant treatment in schizophrenia, some studies used additional items besides the scales. Two of the studies [24,25] investigated changes in Superoxide dismutase (SOD) levels during treatment. SOD is a critical scavenging enzyme involved in the detoxification of superoxide radicals in schizophrenia. Elevated SOD levels in plasma indicate increased oxidative stress, while decreased SOD levels indicate reduced oxidative stress [24].

Results

Inclusion of studies

The systematic search yielded 14 articles, of which 3 were not written in English, Dutch, German or French. Of the remaining articles, 8 were not included based on the inclusion- and exclusion criteria: 3 articles assessed the use of antioxidants as a treatment for the side effects of antipsychotics, 3 studies did not use psychiatric scales, 1 article studied the use of antioxidants as a therapy for sleeping disorders of schizophrenia patients and 1 article was an extension of an article already included [24].

By searching the references of one of the results, another relevant article was included. This article was not found in the initial search due to the absence of 'antioxidant' as a keyword in the article. Ultimately, 4 articles remained for reviewing (Figure 1, Table 1, added as supplement).

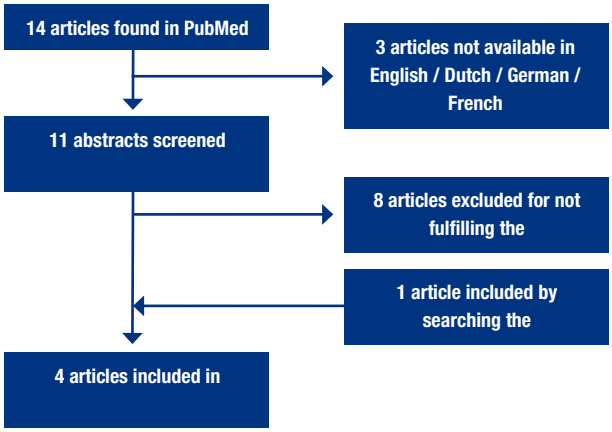


Figure 1 - Flowchart of the search method

	Number of participants (treatment vs. placebo)	Duration of study (weeks)	Mean age (SD)
Zhang et al. (2001)	109 (56 vs. 53)	12	44.2 (7.9)
Atmaca et al. (2005)	29 (15 vs. 14)	8	27.1 (7.3)
Dakhale et al. (2005)	40 (20 vs. 20)	8	36.3 (12.8)
Berk et al. (2008)	140 (69 vs. 71)	24	36.6 (10.9)

SD, standard deviation

Table 1 - Basic information of the analyzed studies

Randomization and blinding

Zhang et al. recruited subjects from the inpatient units of the Beijing Huilongguan Psychiatric Hospital. The study was double-blind, randomized and placebo-controlled. The scores of the scales were assessed by four different clinical psychiatrists.

Atmaca et al. randomly assigned the subjects into two groups, one using olanzapine plus EGb and one using olanzapine only. The study was not placebo controlled. The scores of the scales were assessed by one clinical psychiatrist. The Firat University School of Medicine Department of Psychiatry (Elazig, Turkey) was used as a source of subjects.

Dakhale et al. randomized the subjects and was double-blind and placebo-controlled. One psychiatrist did the assessment and scoring . All patients were from the Outpatient Department of Psychiatry, GMCHNI in Nagpur, India.

Finally, Berk et al. was also a randomized, double-blind and placebo-controlled. Several psychologists and medical practitioners assessed the patients. Participants were recruited through advertisements, referral by clinicians and database screening.

SAPS

All three studies that used the SAPS questionnaire [24-26] as one of the outcome measures reported significant improvement of positive symptoms within the treatment group (mean 4.3 p=0.004, mean -9.4 p<0.05, mean -2.3 p<0.001, Table 2, added as supplement).

Table 2 - Mean difference in outcome measures within treatment and placebo groups, baseline compared with endpoint

	Zhang et al. (2001)				Atmaca et al. (2005)				Dakhale et al. (2005)				Berk et al. (2008)			
	therapy		placebo		therapy		placebo		therapy		placebo		therapy		placebo	
	mean (SD)	P	mean (SD)	P	mean (SD)	P	mean (SD)	P	mean (SD)	P	mean (SD)	P	mean (95% CI)	P	mean (95% CI)	P
SAPS	4.3	0.004	0.7	0.09	-9.4 (1.6)	<0.05	-3.8 (0.8)	<0.05	*	*	*	*	-2.3 (-3.5, -1.1)	<0.001	-1.8 (-2.9, -0.7)	<0.001
SANS	7.1	0.24	5.3	0.051	-3.4 (1.1)	>0.05	-2.7 (0.8)	>0.05	*	*	*	*	-1.6 (-2.7, -0.5)	>0.05	0.24 (-0.8-1.2)	>0.05
BPRS	5.2	0.001	2.7	0.04	*	*	*	*	-14.79 (4.87)	<0.001	-6.93 (4.82)	<0.01	*	*	*	*

SAPS, Scale for Assessment of Positive Symptoms; SANS, Scale for Assessment of Negative Symptoms; BPRS, Brief Psychiatric Rating Scale; SD, standard deviation; CI, confidence interval
* Psychiatric scale not evaluated in the study.

Table 3 - Mean difference in outcome measures between treatment and placebo groups

	Zhang et al. (2001)		Atmaca et al. (2005)		Dakhale et al. (2005)		Berk et al. (2008)	
	mean	p-value	mean	p-value	mean	p-value	mean (95% CI)	p-value
SAPS	3.9	0.026	6.5	<0.05	*	*	0.5 (-1.1, 2.1)	>0.05
SANS	3.3	0.08	-1.5	>0.05	*	*	1.8 (0.3, 3.3)	<0.05
BPRS	2.9	0.058	*	*	9.66	<0.01	*	*

SAPS, Scale for Assesment of Positive Symptoms; SANS, Scale for Assessment of Negative Symptoms; BPRS, Brief Psychiatric Rating Scale; SD, standard deviation; CI, confidence interval. Positive mean is in favor of the therapy group, negative mean is in favor of the placebo group.
* Psychiatric scale not evaluated in the study.

Two of these studies used Ginkgo biloba extract as the antioxidant [24,25], and the third used N-acetyl-cysteine [26]. In two studies [25,26], the SAPS score also improved significantly in the placebo group (mean -3.8 p<0.05, mean -2.3 p<0.001, Table 2). However, substantially more improvement was reported in the treatment groups of two studies [24,25] (mean 3.9 p=0.026, mean 6.5 p<0.05, Table 3).

In Zhang et al., Ginkgo biloba was also assessed as an antioxidant treatment based on clinical effectiveness. Significantly more responders were found in the treatment group than in the placebo group (57% vs. 38%, p=0.043).

SANS

SANS was used in three studies [24-26], but none showed significant improvement within the groups (Table 2). One study reported a significant improvement in the treatment group relative to the placebo group [26] (mean 1.8 p<0.05, Table 3).

BPRS

BPRS was used in two of four studies [24,27]. In both of these studies the treatment group scored significantly better on the scale after antioxidant therapy (mean 5.2 p=0.001, mean -14.79 p<0.001, Table 2). The BPRS score also improved significantly in both placebo groups (mean 2.7 p=0.04, mean -6.93 p<0.01, Table 2). However, more improvement was reported in the treatment group relative to the placebo group in one of the studies [27] (mean 9.66 p<0.01, Table 3).

Other outcome measures

Berk et al. also compared the treatment group and placebo group using the Clinical Global Impression-Severity (CGI-S) scale. This showed a significant mean difference in favor of the treatment group (-0.26, 95% CI (-0.44, -0.08), p=0.004). The treatment group also improved significantly compared to the placebo group, based on the CGI scale (-0.22, 95% CI (-0.41, -0.03), p=0,035).

Zhang et al. and Atmaca et al. also studied changes in SOD levels. They found that these levels were elevated at baseline in schizophrenia patients in comparison to healthy controls (in ng/mg Hb, 815.8 ± 697.8 vs. 515.8 ± 70.4, p<0.05). Atmaca et al. confirmed this finding (in U/g Hb, 1211.1 ± 234.2 vs. 961.6 ± 100.2, p<0.05). Zhang et al. concluded that these levels decrease significantly in schizophrenia patients who receive antioxidant treatment relative to placebo (in ng/mg Hb, 596.7 ± 148.3 vs. 617.6 ± 189.7, p<0.05). Atmaca et al. did not confirm this significance (in U/g Hb, 986.4 ± 134.2 vs. 1189.9 ± 284.8, p>0.05).

Discussion

Although in every study a significant improvement was found in on a least one of the scales (PANSS, BPRS), while analyzing the results we did not find overwhelming proof of the effectiveness of antioxidant use together with antipsychotic drugs (Table 4). SAPS was found to be significantly improved in two [24,25] of three [24-26] publications. Consequently, positive symptoms appear to be most affected by antioxidant treatment. In contrast, SANS was reported to be significantly improved in only one [26] of three [24-26] studies, implying less effect on negative symptoms.

BPRS was studied in two trials [23,26], one of which reported significant improvement [27], while the other did not [24]. This also implies possible effectiveness of antioxidants in improving symptoms occurring in schizophrenia patients.

CGI-S and CGI-I both showed significant improvement in symptoms in the treatment group relative to the placebo group [26]. This would favor the use of antioxidants as a supplementary treatment to diminish schizophrenia symptoms. However, CGI is considered a less reliable scale, since the instrument is based on the previous experiences of investigators [31]. We believe that this compromises the reliability of the results.

SOD levels appear to be elevated at baseline in schizophrenia patients, as reported in two studies [24,25]. This could indicate that oxidative stress plays a role in the pathophysiology of schizophrenia. One of the studies [24] concluded that the use of antioxidants may result in lower SOD levels, and could therefore reduce oxidative stress in schizophrenia patients. However, further investigation is needed.

Limitations

The reliability of a review increases when more studies are included. Therefore, one of the major limitations of this review is that it is based on only four articles. Unfortunately, we found no additional studies on this topic. Another limitation, which every review is confronted with, is that the search strategy is never complete. We may have missed some relevant articles. Finally, we only used one database (PubMed) to find our articles.

The articles we reviewed also had limitations. Two of the four studies had a small sample size [25,27]. Since studies with a small number of participants with non-significant results are less likely to be published, publication bias may have occurred [32]. This could negatively affect the credibility of the review. In addition, tthe studies used relatively subjective assesment scales only to determine the changes in patients, and did not include more objective instruments such as brain scans or EEGs.
Although the studies took a number of confounders into consideration (e.g. smoking, alcohol), other confounders could still have influenced the results (e.g. diet, stress hormones).

Table 4 - Overview results

	Significant improvement	Non-significant result
SAPS	2	1
SANS	1	2
BPRS	1	1

Another limitation was the fact that the articles did not give any information about the type of schizophrenia studied. The response to antioxidants may vary between different types of schizophrenia. Furthermore, all studies except Atmaca et al. included subjects with a relatively advanced age at baseline. It is known that older patients usually experience more negative symptoms [33] in contrast to younger ones, of which the majority suffer from positive symptoms. Since antioxidants seem to have more effect on positive symptoms, the results could have been more significant with a younger patient group. Also, because it would be unethical to ask participants to stop using their medication for research purposes, all included studies examined the effect of antioxidants supplementation along with antipsychotics. This could have affected the results.

Finally, only two out of four articles included a baseline table containing the possible confounders.

Conclusion

The results of our study suggest that antioxidant supplementation may decrease symptoms in schizophrenia patients. Especially those suffering from positive symptoms could benefit from this treatment. If further research provides credible evidence of the benefits of antioxidants in schizophrenia patients, then this treatment could be easily and inexpensively combined with current therapy. We also suggest further research to identify the responsive subgroup of patients, determine the most effective antioxidant and to specify the cost-effectiveness of this treatment option.

References

1. *van Os J, Kapur S. Schizophrenia. Lancet. 2009; 22: 635-645.*
2. *Leucht S, Corves C, Arbter D, et al. Second-generation versus first-generation antipsychotic drugs for schizophrenia: a meta-analysis. Lancet. 2009; 3: 31-41.*
3. *Kane JM. Risperidone: new horizons for the schizophrenic patient. J Clin Psychiatry. 1994; 55: 3-4.*
4. *Stadtman ER. Protein oxidation and aging. Free Radic Res. 2006; 40: 1250-1258.*
5. *Kuloglu M, Ustundag B, Atmaca M, et al. Lipid peroxidation and antioxidant enzyme levels in patients with schizophrenia and bipolar disorder. Cell Biochem Funct. 2002; 20: 171-175.*
6. *Bilici M, Efe H, Koroglu MA, et al. Antioxidative enzyme activities and lipid peroxidation in major depression: alterations by antidepressant treatments. J Affect Disord. 2001; 64: 43-51.*
7. *Jacobs BP, Browner WS. Ginkgo biloba: a living fossil. Am. J. Med. 2000; 108: 341-342.*
8. *DeFeudis FV. Coronary atherosclerosis: current therapeutic approaches and future trends. Life Sci. 1991; 49: 689-705.*
9. *Maclennan KM, Darlington CL, Smith PF. The CNS effects of Ginkgo biloba extracts and ginkgolide B. Prog. Neurobiol. 2002; 67: 235-257.*
10. *Puebla-Perez AM, Lozoya X, Villasenor-Garcia MM. Effect of Ginkgo biloba extract, EGb 761, on the cellular immune response in a hypothalamic-pituitary-adrenal axis activation model in the rat. Int. Immunopharmacol. 2003; 3: 75-80.*
11. *Tian YM, Tian HJ, Zhang GY, et al. Effects of Ginkgo biloba extract (EGb 761) on hydroxyl radical-induced thymocyte apoptosis and on age-related thymic atrophy and peripheral immune dysfunctions in mice. Mech. Ageing Dev. 2003; 124: 977-983.*
12. *Sauberlich HE. Pharmacology of vitamin C. Annu. Rev. Nutr. 1994; 14: 371-391.*
13. *Oke AF, May L, Adams RN. Ascorbic acid distribution patterns in human brain. A comparison with nonhuman mammalian species. Ann. NY Acad. Sci. 1987; 498: 1-12.*
14. *Meister A, Anderson ME. Glutathione. Annu. Rev. Biochem. 1983; 52: 711-760.*
15. *Do KQ, Trabesinger AH, Kirsten-Kruger, et al. Schizophrenia: glutathione deficit in cerebrospinal fluid and prefrontal cortex in vivo. Eur. J. Neurosci. 2000;12: 3721-3728.*
16. *Tosic M, Ott J, Barral S, Bovet P, et al. Schizophrenia and oxidative stress: glutamate cysteine ligase modifier as a susceptibility gene. Am. J. Hum. Genet. 2006; 79: 586-592.*
17. *Gysin R, Kraftsik R, Sandell, et al. Impaired glutathione synthesis in schizophrenia: convergent genetic and functional evidence. Proc. Natl. Acad. Sci. USA. 2007; 104: 16621-16626.*
18. *Sjodin K, Nilsson E, Hallberg A, et al. Metabolism of N-acetyl-L-cysteine. Some structural requirements for the deacetylation and consequences for the oral bioavailability. Biochem. Pharmacol. 1989; 38: 3981-3985.*
19. *Holdiness MR. Clinical pharmacokinetics of N-acetylcysteine. Clin. Pharmacokinet. 1991; 20: 123-134.*
20. *Leucht S, Kane JM, Etschel, et al. Linking the PANSS, BPRS, and CGI: clinical implications. Neuropsychopharmacology. 2006; 31: 2318-2325.*
21. *Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr. Bull. 1987; 13: 261-276.*
22. *Leucht S, Kane JM, Kissling W, et al. Clinical implications of Brief Psychiatric Rating Scale scores. Br. J. Psychiatry. 2005; 187: 366-371.*
23. *Haro JM, Kamath SA, Ochoa S, et al. The Clinical Global Impression-Schizophrenia scale: a simple instrument to measure the diversity of symptoms present in schizophrenia. Acta. Psychiatr. Scand. Suppl. 2003; 416: 16-23.*
24. *Zhang XY, Zhou DF, Zhang PY, et al. A double-blind, placebo-controlled trial of extract of Ginkgo biloba added to haloperidol in treatment-resistant patients with schizophrenia. J. Clin. Psychiatry. 2001; 62: 878-883.*
25. *Atmaca M, Tezcan E, Kuloglu M, et al. The effect of extract of ginkgo biloba addition to olanzapine on therapeutic effect and antioxidant enzyme levels in patients with schizophrenia. Psychiatry Clin. Neurosci. 2005; 59: 652-656.*
26. *Berk M, Copolov D, Dean O, et al. N-acetyl cysteine as a glutathione precursor for schizophrenia--a double-blind, randomized, placebo-controlled trial. Biol. Psychiatry. 2008; 64: 361-368.*
27. *Dakhale GN, Khanzode SD, Khanzode SS, et al. Supplementation of vitamin C with atypical antipsychotics reduces oxidative stress and improves the outcome of schizophrenia. Psychopharmacology. 2005; 182: 494-498.*
28. *Quintero J, Barbudo del Cura E, Lopez-Ibor MI, et al. The evolving concept of Treatment- Resistant Schizophrenia. Actas. Esp. Psiquiatr. 2011; 39: 236-250.*
29. *Ranjekar PK, Hinge A, Hegde MV, et al. Decreased antioxidant enzymes and membrane essential polyunsaturated fatty acids in schizophrenic and bipolar mood disorder patients. Psychiatry. Res. 2003; 121: 109-122.*
30. *Boskovic M, Vovk T, Kores Plesnicar B, et al. Oxidative stress in schizophrenia. Curr Neuropharmacol 2011; 9: 301-312.*
31. *Forkmann T, Scherer A, Boecker M et al. The clinical global impression scale and the influence of patient or staff perspective on outcome. BMC Psychiatry. 2011; 11: 83.*
32. *Sterne JA, Gavaghan D, Egger M. Publication and related bias in meta-analysis: power of statistical tests and prevalence in the literature. J. Clin. Epidemiol. 2000; 53: 1119-1129.*
33. *Kelley ME, Haas GL, van Kammen DP. Longitudinal progression of negative symptoms in schizophrenia: a new look at an old problem. Schizophr. Res. 2008; 105: 188-196.*

Smokers should not cough up a higher health insurance contribution

Marianne A. Langhout
Medical student, Erasmus MC University Medical Center Rotterdam, the Netherlands
Correspondence: Marianne A. Langhout, email: m.langhout@erasmusmc.nl

Introduction

For many years, public health policy in the Netherlands has aimed at reducing the prevalence of smoking. Many smokers know that their smoking behavior can cause serious health damage, partly due to the mandatory warnings on cigarette packages. The anti-smoking campaigns in the Netherlands seem to be effective, as shown by the lower smoking prevalence over the last decade [1]. Still, the prevalence of smoking in the Netherlands is among the highest in Europe [2]. To lower the number of smokers, the Dutch government is searching for new methods to make smoking less appealing. A controversial standpoint, which has recently acquired more support, is to link smoking behavior to the health insurance contribution. Smoking, as an exogenous risk factor, is held responsible for a high disease burden and increased healthcare costs. Therefore, according to this standpoint, smokers should pay more for their health insurance than non-smokers. Besides providing financial benefits for health insurers, this policy could potentially reduce smoking prevalence.

This proposed lifestyle-dependent contribution has received a broad range of reactions. When making such a statement, lots of questions arise, but the answers seem to be ambiguous so far. The subgroup of smokers comprises more than a quarter of the total Dutch population [3]. Therefore, introducing a health insurance policy, which links lifestyle factors to health insurance contribution, could affect many Dutch citizens.

In this essay, the controversy surrounding a smoking-behavior-dependent health insurance contribution is analyzed. Numerous aspects of this topic are addressed, leading to the conclusion that the health insurance contribution should not depend on the smoking behavior of the insured.

Thesis

To reach a valid conclusion, the assumed higher burden of disease due to smoking, and its related costs to society must be quantified. Another supposed advantage of the policy is the expected decrease in smoking prevalence that would result. It is crucial to clarify whether linking health insurance contribution to smoking behavior is related to smoking prevalence, and how.

Moreover, the ethical aspects of this policy, such as its lack of fairness, need to be evaluated. Is this distinction a form of discrimination, or is it an appeal to individual responsibility? In addition, does this policy violate the autonomy of smokers?

The answers to these questions will help clarify the issues surrounding the thesis of this essay: the health insurance contribution should depend on the smoking behavior of the insured.

A scientific view

The adverse health effects of smoking are undisputed [4,5]. Nevertheless, many Dutch citizens continue to smoke: in 2009 the prevalence of smoking was 27.1% [3]. But what are the actual costs to society due to smoking?

Research by RIVM (the Dutch National Institute for Public Health and the Environment) shows that smoking is responsible for more loss in healthy life years than other exogenous factors. Smokers show a life expectancy reduction of four years, and a loss of almost five healthy life years [6]. This makes smoking the determinant that contributes most to the total number of DALYs (Disability Adjusted Life Years), namely 13.0% [7]. DALYs are calculated by adding YLL (Years Life Lost) to the loss of healthy life years. The same research shows that smoking is responsible for 3.7% of the average annual cost of disease [7].

These studies clearly illustrate the increase in short-term health care costs due to smoking. Many studies and articles have reached the same conclusion. However, it is an oversimplification to conclude that smoking increases overall health care costs. The discord, which seems to be inherent to the debate on smoking and its costs, is caused by studies that show a decrease in long-term healthcare costs due to smoking. Research has shown that smoking cessation reduces short-term healthcare costs, but it also increases life expectancy, which in turn increases long-term demand for healthcare. These additional life years due to smoking cessation cost society more than the savings on short-term healthcare costs [8,9]. These surprising results suggest that continued smoking actually decreases long-term healthcare costs for society compared to stopping smoking.

The previously mentioned cost-analyses only involved smoking related costs in healthcare. However, smoking can also be responsible for other costs to society. PricewaterhouseCoopers performed a cost-efficiency analysis on prevention of unhealthy lifestyles, which showed that young smoking citizens cost Dutch society about €2.4 billion. The 2.4 billion Euros are based on costs of total productivity loss over a working lifetime if current 15-20 year old smoking workers continue smoking. They tend to have lower productivity due to more disease and are less efficient compared to their non-smoking equivalents [10]. The benefits from added life years through smoking cessation were included in the analyses. According to this more comprehensive study, smokers cost society more than non-smokers, mostly due to their lower productivity.

If changes in smoking behavior due to increasing the health insurance contribution respond similarly to increasing the excise taxes on tobacco, the intervention will have little effect on decreasing smoking prevalence [10].

Ethical aspects

The debate on introducing a smoking-behavior-dependent health insurance contribution involves conflicting ethical principles, such as the right to autonomy and self determination as opposed to the obligation of the government to protect its citizens. Also, the appeal to individual responsibility is debatable.

The amended policy makes an inter-individual distinction based on smoking behavior. If the consequences of this distinction are fair, then it does not necessarily lead to inequity. For example, holding smokers responsible for the actual consequences of their behavior would seem to be logical and fair. However, by introducing this policy, smokers would pay more health insurance contribution, even though their lifestyle results in lower healthcare costs in the long term. Making smokers pay for expenses in healthcare for which they are not responsible, but even reduce, violates the theory of justice [11]. Moreover, the amended health insurance policy violates autonomy. Freedom of choice and self determination are fundamental to autonomy [11]. In case of optimal autonomy, a choice should not be influenced by others. Generally, smokers are competent enough to make well thought out decisions concerning their smoking behavior and can be held responsible for their own health.

However, the argument of competence conflicts with the obligation of the government to protect its citizens. Although patient competence makes this obligation less important, Article 22 of the Dutch constitution states the government must take measures to pursue improvement of public health [12]. In my opinion, Article 22 does not apply to this change in health insurance policy. This policy is not so much about improving public health, but aims for healthcare cost reduction.

Argumentation

Based on the evidence presented above, a smoking-behavior-dependent health insurance contribution is an inappropriate and unfair policy. The increased costs to society due to smoking are located outside the health care sector, and should not be compensated by increasing the health insurance contribution. Besides, smokers are already being held responsible for increase in costs to society through excise taxes on tobacco. Discriminating against smokers regarding health insurance contribution cannot be justified because they cannot be held responsible for increasing healthcare costs in the long term.

Furthermore, smoking is not the only potential cost increasing, exogenous risk factor [7]. What about other risk factors, such as insufficient exercise, excessive drinking and poor diet? A consistent policy should take all high risk lifestyle factors into account.

But executing such a policy can lead to problems. People are less likely to admit they have a high risk profile if this leads to higher costs. Identifying high risk lifestyle factors in the insured would cost a great amount of money, and may even be impossible.

Finally, access to healthcare should not be restricted for any reason. Even reproachable behavior should not impede access to adequate health care.

Feeling save by security of the body and health is a basic level in Maslov's hierarchy of human needs [13]. Open access to healthcare improves this feeling of security.

Conclusion

Smoking does not increase long-term healthcare costs. Multiple ethical objections accompany an introduction of a smoking-behavior-dependent health insurance contribution, such as unfairness, violation of autonomy and impeding access to healthcare. Therefore, the health insurance contribution should not depend on the smoking behavior of the insured.

References

1. *STIVORO. Percentage rokers Nederlandse bevolking 1958-2010. 2010; Available from: http://www.stivoro.nl/Upload/Trendpublicatie%20Percentage%20Rokers%202010%20%282%29.pdf*
2. *Nationaal Kompas Volksgezondheid. Zijn er verschillen tussen Nederland en andere landen? 2011; Available from: http://www.nationaalkompas.nl/gezondheidsdeterminanten/leefstijl/roken/verschillen-internationaal/*
3. *CBS StatLine. Gezondheid, leefstijl, gebruik van zorg. 2010; Available from: http://statline.cbs.nl/StatWeb/publication/?DM=SLNL&PA=03799&D1=202- 209&D2=0-17&D3=0&D4=a&VW=T*
4. *Thun MJ, Apicella MF, Henley SJ. Smoking vs other risk factors as the cause of smoking-attributable mortality: confounding in the courtroom. JAMA. 2000; 284: 706-712.*
5. *Ezatti M, Lopez AD. Estimates of global mortality attributable to smoking in 2000. Lancet. 2003; 9387: 847-52.*
6. *Nationaal Kompas Volksgezondheid. Ziektelast in DALY's samengevat. 2010; Available from: http://www.nationaalkompas.nl/gezondheid-en-ziekte/sterfte-levensverwachting-en-daly-s/ziektelast-in-daly-s/ziektelast-in-daly-ssamengevat/*
7. *RIVM. Welke factoren bepalen onze gezondheid? De bijdrage van determinanten aan de ziektelast. 2006; Available from: http://www.rivm.nl/vtv/object_document/o5437n30048.html*
8. *Barendregt JJ, Bonneux L, Van der Maas PJ. The health care costs of smoking. N Engl J Med. 1997; 15: 1052-57.*
9. *Brouwer WBF, Van Exel NJA, Polder JJ et al. Gezond leven niet per se goedkoper. Medisch Contact. 2006; 21: 878-81.*
10. *PricewaterhouseCoopers. Project "Prevention Pays for Everyone" Return on investment in a healthier lifestyle. Prevent Conference, Oegstgeest. 2010.*
11. *Hilhorst M, Schermer M. Vier ethische basisprincipes. Voorbereiding Bachelor Essay. Erasmus MC 2010-2011.*
12. *Nederlandse Grondwet. Hoofdstuk 1 Grondrechten, artikel 22.1 Volksgezondheid. Available from: http://www.denederlandsegrondwet.nl/9353000/1/j9vvihlf299q0sr/vgrnbac43qvy#p22*
13. *Maslow, A.H. A Theory of Human Motivation. Psychological Review. 1943; 4: 370-96*

Female genital cosmetic surgery: about patient choice and protection

Juliette van Kalmthout[a,b], BA, BSc
[a] Medical Student, Erasmus University Medical Center, Rotterdam, the Netherlands
[b] Research Scholar, Yale University School of Medicine, New Haven, Connecticut, USA
Correspondence: Juliette van Kalmthout, e-mail: juliette.vankalmthout@yale.edu

Cosmetic trends

In 2007, Sunny Bergman produced the Dutch documentary *Beperkt houdbaar* [1] about the aesthetic ideals and norms of women that may be influenced by the coercive effects of advertising [1-12]. Bergman questions whether the idealized image of the female body in the media is healthy and feasible. Also in 2007, the VARA network broadcast the Zembla documentary *Borsten voor je verjaardag*. This program could be interpreted as a warning of the harmful risks of cosmetic surgery. The main storyline in this documentary focused on Marion, a 21 year old woman who died from complications after a liposuction procedure [11]. Both TV documentaries referred to the increasing popularity of female genital cosmetic surgery.

The hairless vulva seems to be quite popular these days. The trend began in the pornography industry and has since become integrated into Western society [2,5,7,8,10,12]. This fashion concept of making the vulva more visible may contribute to the rise of a new genital aesthetic ideal [2,5,10]. Women reportedly undergo female genital cosmetic surgery because they want a "tidy" vulva that resembles that of a prepubescent girl [1,2,5]. But should the plastic surgeon routinely approve these requests? Or should female genital cosmetic surgery be prohibited by law since it could be hazardous?

Facts about female genital cosmetic surgery

Female genital cosmetic surgery includes labia minora reduction, labia majora augmentation, vaginal tightening (also called vaginal rejuvenation), pubic liposuction, clitoral hood reduction, hymen reconstruction, perineum rejuvenation, and G-spot amplification [2,4,9,10,13]. The most common procedure is labia minora reduction [5,8,14].

Most women who seek female genital cosmetic surgery do so for aesthetic and/or functional reasons [2,5]. The aesthetic reasons primarily concern the visibility of the labia minora or their shape, color or asymmetry. The functional reasons include vaginal "laxity" during intercourse or irritation of the labia when exercising or wearing tight clothing. Also, psychological concerns motivate women to seek female genital cosmetic surgery. These include embarrassment in sexual or social settings [2,4,5,8,9,11,13]. Apparently, contemporary culture prefers smaller labia [5,8].

The costs for female genital cosmetic surgery in the Netherlands range between €1000 and €3000. In general, health insurance does not reimburse the costs. According to Dutch law, persons over 16 are eligible to have cosmetic surgery. Under the age of 16, permission of both parents is required [15]. According to the guidelines of the Dutch Association for Plastic Surgery (NVPC), cosmetic surgery patients must be over 18 [16]. Nevertheless, plastic surgeons who are not NVPC-certified do not necessarily follow these guidelines [15].

Patient choice

Assuming they can afford it, Dutch women can easily obtain genital cosmetic surgery [1,11]. Should women be protected from these procedures by law? I believe that prohibition of female genital cosmetic surgery is at odds with medical ethics, specifically the principle of patient autonomy. A young woman who is unhappy about the appearance of her vulva has the right to try and change it [3,5,9,12]. The only requirement is that she must be mentally competent to make such a decision. Therefore, a psychological consultant should determine if she is suffering from a depression, anxiety or body dysmorphic disorder [3,5,17]. Also, the woman must be fully aware of the risks of the surgical procedure [2-5,7,8]. If a woman is found to be mentally competent, if she understands the risks and still wants the surgery, she should be able to have it done [3,11]. Consenting adults should be left to make their own choices [9,12].

Influenced autonomy?

Some critics make the following objection: how can autonomous choices be made when society and media put pressure on women to alter their appearance [1-11]? They think that women's autonomy is influenced by sexist beauty standards [6,7,9,12], and that their norms are manipulated by "Photoshopped" images of vulvas. These marketing images are misleading because they do not show the enormous anatomic variation in female genitals [2,7,8].

I disagree with the statement that media pressure prevents autonomous decision-making. Some women deliberately abide by these so-called imposed beauty standards. They want to be stylish [6,11]. They understand that the vulvas shown in magazines are not realistic. A body with tattoos and piercings is not a realistic image either. Some women see their piercings as markers of their individuality, or as ritually empowering or celebratory [9,12]. Such women gain self-confidence by choosing their own appearance [6,9,12].

However, other women are negatively affected by the commercial images of vulvas. These more vulnerable women do not understand that these images are unrealistic. They request surgery with the aim of "finally becoming normal" [2,5,9,12]. Therefore, I believe that female genital cosmetic surgery should be performed only after a psychological consultation [5,12]. During this consultation, images should be shown that clearly indicate the wide anatomic variation between vulvas [3,5]. The women should be informed about the normality of these variations [3,5,17]. Also, the consultant can determine if the patient is psychologically stable and realistic in her expectations [5,12].

Female genital cosmetic surgery vs. female genital mutilation

In the literature, female genital cosmetic surgery has often been compared with female circumcision [6,9,10,12]. If we only focus on what in the anatomy is removed, the modifications are indeed comparable [10]. But in other ways it is not comparable.

The vast majority of female circumcisions are performed on juveniles and infants with no regard for their wishes [10,12]. If an adult woman in certain cultures is uncircumcised, she may be considered to be unmarriageable or could be disinherited. Consequently, uncircumcised adult women are often compelled to be circumcised due to family and religious pressure [9,10,12]. In other words, these women are not free to choose.

In the case of female genital cosmetic surgery, women are free to choose – as long as they are mentally competent and psychologically stable [10,12]. They will not face comparable consequences if they are not operated. Whether inspired by contemporary beauty ideals or not, the decision about the surgery is therefore entirely autonomous [10,12].

First, do no harm

Regarding the surgery itself, the ethical principles of non-malfeasance and beneficence must be strongly considered [3,5]. Female genital cosmetic surgery, like all surgery, entails the risk of medical complications [10,11]. Reported complications include bleeding, infection, altered genital sensitivity, pain during intercourse, adhesions and scarring [2,5,14]. Some studies suggest that female genital cosmetic surgery has a high rate of patient satisfaction [3,12,14,17,18], while other studies conclude that not enough research with adequate follow-up has been conducted to support this assertion. According to these studies, long-term sexual function and patient satisfaction have not been thoroughly studied [2,3,7,17]. Because patients who choose cosmetic surgery are usually healthy, exposing these women to risks of complications and unclear long-term effects could be viewed as malfeasance.

Of course, "First, do no harm" must be taken seriously [2,5,14]. But is prohibiting the surgery then the right option? After watching the documentaries *Borsten voor je verjaardag* [11] and *Beperkt houdbaar* [1], I concluded that the interviewed women were very certain that they wanted female genital cosmetic surgery. Prohibiting such surgery could therefore lead to illegal surgical clinics. Non-certified surgeons working clandestinely would almost certainly have higher complication rates, thus increasing the risk. The best solution for these women would be treatment by NVPC-certified plastic surgeons working in a monitored clinic using evidence-based surgical techniques [8,14]. The partial legalization of soft drugs in the Netherlands is based on the same principle: harm reduction. This makes the sale of soft drugs controllable and therefore safer [19].

Paternalism

According to plastic surgeon Martin Janssen, who was interviewed in *Borsten voor je verjaardag* [11], refusing women to have genital cosmetic surgery is paternalistic. Janssen works at the Boerhaave clinic in Amsterdam and is NVPC-certified. "A medical doctor should first listen to the wishes of the patient.", he says. "You don't take the doctor-patient relationship seriously if you ignore the wishes of a patient just because you want to protect her." The protective function of the physician should not overrule the wishes of the patient; this is a carry-over from earlier times in medicine. Medical practice has developed within norms that are bound by time and culture [6,10,12].

Conclusion

Female genital cosmetic surgery should not be prohibited by law. Prohibition denies the right of autonomy. However, certain preconditions are necessary. An extensive psychological assessment should be mandatory beforehand. Women who are considering surgery should be informed about the normal range of genital anatomic variation. After these steps, the surgeon is then responsible for informing women about all the risks of the procedure. The procedure should be performed only if the candidate is fully informed, psychologically stable and mentally competent. A paternalistic doctor-patient relationship is not suitable for this time. The autonomy of the patient is central. An informed woman can decide for herself what she wants to do with her body. She may decide to have a breast augmentation, to start smoking, to eat unhealthy food, to get piercings or to go bungee jumping. The Netherlands is a free country with a free society. We should be proud of that, as proud as we may be of our self-determined modified designer vulva.

References

1. *Bergman S. Beperkt houdbaar. Holland Doc. 2007.*
2. *Braun V. Female genital cosmetic surgery: a critical review of current knowledge and contemporary debates. J Womens Health. 2010; 19: 1393-407.*
3. *Paarlberg KM, Weijenborg PT. Request for operative reduction of the labia minora; a proposal for a practical guideline for gynecologists. J Psychosom Obstet Gynaecol. 2008; 29: 230-4.*
4. *Liao LM, Michala L, Creighton SM. Labial surgery for well women: a review of the literature. BJOG. 2009; 117: 20-5.*
5. *Dobson J. Understanding female genital cosmetic surgery. BMJ. 2012; 345: e4819.*
6. *Schermer M. Minima philosophica: labiaplastiek. In: De Kwestie. 2 ed. Den Haag: Boom Lemma uitgevers. 2011. p. 366-9.*
7. *Michala L, Koliantzaki S, Antsaklis A. Protruding labia minora: abnormal or just uncool? J Psychosom Obstet Gynaecol. 2011; 32: 154-6.*
8. *Liao LM, Creighton SM. Female genital cosmetic surgery: a new dilemma for GPs. Br J Gen Pract. 2011; 61: 7-8.*
9. *Kelly B, Foster C. Should female genital cosmetic surgery and genital piercing be regarded ethically and legally as female genital mutilation? BJOG. 2012; 119: 389-92.*
10. *Johnsdotter S, Essen B. Genitals and ethnicity: the politics of genital modifications. Reprod Health Matters. 2010; 18: 29-37.*
11. *Blaas M. Borsten voor je verjaardag. VARA. Zembla. 2007.*
12. *Dorneles de Andrade D. On norms and bodies: findings from field research on cosmetic surgery in Rio de Janeiro, Brazil. Reprod Health Matters. 2010; 18: 74-83.*
13. *Mirzabeigi MN, Jandali S, Mettel RK et al. The nomenclature of "vaginal rejuvenation" and elective vulvovaginal plastic surgery. Aesthet Surg J. 2011; 31: 723-4.*
14. *Scholten E. Female genital cosmetic surgery--the future. J Plast Reconstr Aesthet Surg. 2009; 62: 290-1.*
15. *NOS. HYPERLINK "http://www.nos.nl" www.nos.nl. 2012; April, 12th.*
16. *Nederlandse Vereniging voor Plastische Chirurgie. HYPERLINK "http://www.nvpc.nl" www.nvpc.nl. 2012.*
17. *Likes WM, Sideri M, Haefner H et al. Aesthetic practice of labial reduction. J Low Genit Tract Dis. 2008; 12: 210-6.*
18. *Tepper OM, Wulkan M, Matarasso A. Labioplasty: anatomy, etiology, and a new surgical approach. Aesthet Surg J. 2011; 31: 511-8.*
19. *Ooyen-Houben MMJ. Hoe werkt het Nederlandse drugsbeleid? Justitiële Verkenningen. 2006; 32: 188.*

Abstract

Colchicine in FMF: effects on fertility and pregnancy

Tim Both [a], Jan A.M. van Laar [b], P. Martin van Hagen [b], Femke Bonte-Mineur [c], Paul L.A. van Daele [b]
[a] Medical student, Dept. Immunology, Erasmus MC University Medical Center Rotterdam, the Netherlands
[b] Supervisor, Dept. Immunology, Erasmus MC University Medical Center Rotterdam, the Netherlands
[c] Dept. Reumatology, Maastad Hospital Rotterdam, Erasmus MC University Medical Center Rotterdam, the Netherlands
Correspondence: Tim Both, email: t.both.1@erasmusmc.nl

Objective
Familial Mediterranean fever (FMF) is a periodic disease characterized by recurrent attacks of fever accompanied by peritonitis, pleuritis, artritis, or erysipelas-like skin lesions. Colchicine is the drug of choice for FMF because it controls the acute attacks and prevents the development of amyloidosis. In case reports the use of colchicine has been associated with secondary infertility. We performed a literature search for the possible development of infertility in patients (both men and women) with FMF, on treatment with colchicine.

Design
Systematic literature search

Method
PubMed was searched for English articles with the combination of search criteria, 'colchicine', 'fertility' and 'familial mediterranean fever' and the combination 'colchicine', 'pregnancy' and 'familial mediterranean fever'. The selected articles were reviewed for crossreferences and co-authors were asked for missing articles. All articles describe the effects of colchicine on fertility in animals and/or humans in patients with FMF.

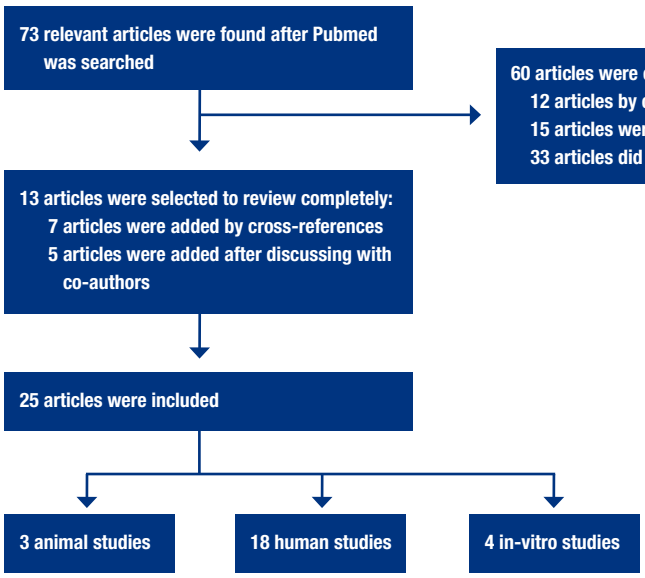
Results
Our search led to seventy-three articles, of which thirteen articles matched our inclusion criteria. We selected another seven articles via cross references. After evaluation with the co-authors, we included another five articles. This result in a total of twenty-five articles (see figure 1). Three of the included articles were animal studies. Four articles contained in-vitro data.

From these articles it appeared that colchicine inhibits the clinical symptoms of FMF and the development of amyloid deposits. No statistically significant effect was found of colchicine treatment on semen quality or hormone levels. Treatment with colchicine during pregnancy did not lead to severe complications. Less severe side effects of colchicine treatment, like diarrhoea, are common. Both male and female patients who were treated with colchicine had a better prospect of maintaining fertility, compared with patients without this treatment.

Conclusion
Literature search revealed that colchicine use in large patient groups has no documented effects on fertility. Untreated FMF itself can lead to amyloid depositions in testes and the ovary, resulting in infertility. We advise to continue colchicine treatment in FMF-patients when planning procreation.

References
Both, T, Bonte-Mineur, F, van Daele, P.L.A. et al. Colchcine in FMF: effects on fertility and pregnancy. Ned. Tijdschr Geneeskd. 2012; 156: A4196

Figure 1



Instructions for EJM authors

General

The instructions that follow have several purposes. First, we want to make life easy for you, the authors, and for the editors and peer reviewers, the layout (prepress) people, and the journal readers.

The Guidelines for the Storyline will help you to organize your article in a logical, credible and readable way. This will help you - it tells you what goes where—and, thus, save you time. It will help the editors and peer reviewers—they will easily see the credibility and relevance of your work— and, thus, save them from writing rejection letters. And, it will help readers to quickly and easily read and understand your work and see its value.

The section entitled Formatting Instructions will help you as well; the basic idea is to keep the formatting as simple as possible, so you can focus on content and not get involved with layout. The language editor and the prepress people will also be able to more efficiently do their jobs. Please follow these instructions.
Please be aware that we will have to return papers that do not conform to these instructions to the authors.

What you can enter

Research articles - Research articles describe one study or analysis, usually from a fourth-year elective research project or one of the masters programs. Number of words: max. 3500 + 4 figures or tables.
Extended abstracts - Extended abstracts consist of a condensed presentation of complete final or temporary results of a study. Number of words: 350 words + 1 figure or table.
Reviews - Second year students can submit their review written in the second year elective course. Number of words: 1500 + 3 figures or tables.
Opinion papers - These are papers that reflect the opinion of the author on a scientific topic. The author should be clear where evidence ends and personal opinion starts. A paper typically has a length of about 1000 words.
Comments - In this section editors, or faculty staff, as well students are invited to write a short critical comment on a paper, putting it into perspective for a broader medical public readership. Number of words: 350.
Letters to the editor - The editorial board encourages students to write a letter to the editor to comment on published papers, or on the journal in general. These will be published on the website of the journal. Letters should not exceed 200 words and may be abbreviated by the editor.
Research projects - Here researchers or teachers describe ongoing research projects at the Erasmus MC University Medical Centre for which they want to invite students to participate. Number of words: 350.

The review process

Papers may be submitted to the editorial office. Please indicate which author will act as corresponding author. We expect this author to maintain contact with the other authors and to speak and decide on their behalf.

Each paper will be assigned to a team consisting of a managing editor and an associate editor. Each submitted paper will be checked for compliance with the author instructions. If this is not the case, the paper may be returned to the author.

When the paper is taken into review, it will be sent out to two external reviewers, a student and a staff member of Erasmus MC. Based upon these reviewers comments, their recommendations and the opinion of the editorial team, a decision will be made: reject, major revision, minor revision, accept with or without minor changes.

The paper will then be returned to the corresponding author, along with the recommendation. We try to return papers within 3 weeks after submission.

When a paper is rejected, it cannot be resubmitted, but we encourage resubmissions when we recommend major or minor changes to a paper. Resubmitted paper will be reviewed again by the same reviewers and editorial team.

Before a paper can be accepted for publication, we will need a statement that the staff member that supervised your work agrees with the submission of your paper. Moreover, we need a signed Copyright Transfer Agreement (CTA) and a signed Conflict of Interest statement. When your research project involves patients or volunteers, we need a statement in the paper that the research protocol has been reviewed by a Medical Ethics Committee. Failure to provide this information at an early stage of the submission may impair the review process.

When a paper is accepted for publication, it will often be forwarded to our language editing and restructuring editors. They will each in turn give recommendations and ask the author adapt the paper accordingly. When this phase is completed, the paper will be forwarded to the publisher. Page proofs will be sent to the author for a final check.

Formatting instructions

Entry format - Papers should be submitted by email, to ejm@erasmusmc.nl. Word 2003 files are preferred for the initial submission. The file should include all figures and tables.
Title page - The title page should clearly identify the authors, the institute where the research project was carried out, as well as the staff member who supervised the project. The corresponding author name (first name and family name), email address, student id, should be clearly indicated. In case of multiple authors, state functions and departments only in superscript in alphabetical order. Example:
First name A.G. Family name[a] and First name W.F. Family name[a]
Supervisor: First name R. Lastname[b]
[a] Medical students, Erasmus MC University Medical Center Rotterdam, the Netherlands
[b] Dept. of Internal Medicine, Erasmus MC University Medical Center Rotterdam, the Netherlands
Correspondence: First name A.G. Family name, email: Firstname-Familyname@me.com.

Structure - Please use the following sections in all papers (except in comments and opinion papers): Abstract, Introduction, Methods, Results, Discussion, References, Tables, Figures.
References - Number references in order of appearance. References should have the following format:
Rothwell, P. M. Medical and surgical management of symptomatic carotid stenosis. Int J Stroke. 2006; 1: 140-149. (I.e. year;vol:ppp-ppp) In case of more than 3 authors, name the first 3 and insert "et al.". Limit the number of references to 30. References should appear in the text as follows: "… treatment is of proven benefit.[1]"
Tables and figures - Tables and illustrations (both numbered in Arabic numerals) should be prepared on separate pages. Number tables and figures separately and consecutively. Tables require a heading and figures a legend, also prepared on a separate page and should be formatted with a text editor (example). Figures should be submitted electronically. B/w half-tone and color illustrations must have a final resolution of 300 dpi after scaling, line drawings one of 800-1,200 dpi (jpg and tiff is an acceptable format). Please note that all color-figures will be converted to gray tones. Please adapt graphs to suit this format, i.e. make use of dotted and dashed lines and hatched bars instead of colored items.. The final submission should contain figures as JPG or TIFF files.

Instructions for EJM authors

Page layout
- Standard margins
- no headers or footers
- no columns
- left align (ragged right)
- font: 12pt Arial
- single line spacing
- main headings 14 pt bold; subheading 12 point italic
- indent every paragraph, except after headings, tables, bulleted lists or figures

Other formatting
- number all tables and figures sequentially
- place tables and figures at the end of article; insert captions at correct locations in body text
- no text boxes
- no footnotes or end notes
- do not submit figures with text as drawing objects (they cannot be edited)
- limit the use of italics and do not use italics for simple emphasis; do not italicize quotations; quotation marks are sufficient
- do not use italics for commonly understood Latin expressions such as "in vitro"
- use italics for other foreign words, such as expressions in Dutch
- no "sub-paragraphs"
- no hyphenation (afbreking)

Language
US English spelling and punctuation

Guidelines for the storyline of a scientific article

Authors of scientific articles often write in a style that was very successful at school but, unfortunately, does not work well in the "real" world. The reason? Readers of scientific journals -"real world readers" - when reading your papers have much different goals than your instructors had. Real world readers do not care how smart you are, or if you have done the homework, and they are not going to "grade" your papers. They do not count words to see if you met the minimum length of the paper. They read journal articles because they are looking for information and ideas that they can use in their own work. In other words, they are looking for something of value. In fact, when you read journal articles you are also a real world reader. Do you care how smart the authors are, or if they did their homework? Probably not. Are you impressed with long complicated sentence constructions and abstract ideas that only seem to fill up space? Do you count words? Probably not.

You probably are impressed, however, when you read an easy to understand article that clearly presents credible and relevant science. You can write that way too. But you need to get "out of the box" of what I call "academic" writing style. These guidelines will help you to get outside the box. The guidelines are based on one very simple concept: give you readers something they can use. Note that this requires a different mode of thinking. At school you probably never thought of giving your instructors something they could use. You wrote to receive something - a good grade. These guidelines will take you step by step through the process of writing a readable scientific article that presents relevant and credible science.

The first step is to write the "storyline" of your article - a readable story that logically ties all of your main ideas together. It focuses on the logical thread that credibly presents the point and the value of your research. It is to be short, logically linked and easily understood by non-specialists. It contains few technical or theoretical details. This requires another shift in your mode of thinking. It requires you to forget, for the moment, the technical and theoretical details and problems that you focus on day-in and day-out. Rather, you need to think in terms of what the reader wants to know - the point and value of your research.

Your storyline will become a "skeleton" for your entire article. After getting the storyline clear, take the second step and add the "muscles" to turn this skeleton into a complete entity - a readable and credible scientific article. The "muscles" are, of course, the technical and theoretical details.

Below, is a short (fictitious) example to illustrate the storyline. In this example, 6 key elements of the standard scientific article form the basic structure of the storyline. You can use this example as a template for your storyline. Under each heading, delete the example text and paste in a similar text about your own work.

Fictitious Example

"Predicting Malaria Epidemics in Ethiopia"

Key element 1: the point of the research - why should the editors and readers care about the study?

'Malaria is still the number one killer of all infectious diseases. Most deaths could be prevented, however, if adequate medical facilities and medicines were available at the beginning of an epidemic. After an outbreak of malaria, getting adequate medical facilities and medicines to the local area can take many weeks. Obviously, this time is truly lost time and, for many victims, fatal. If, however, malaria epidemics could be predicted in local areas, medical facilities and medicine could be mobilized where they will be needed and, thereby, save many lives. Predicting where and when an epidemic can be expected is, however, currently not possible.'

Notice that the above statements clearly present a BIG health-related problem. And, an "if" sentence focuses on a strategy to help solve that problem. In only 3 sentences we know the point, and potential value, of the research. Now it is time to focus on what is known, and prepare the reader to understand the specific research question. For example,

'Malaria epidemics are known to be related to weather conditions. Previous research has shown that malaria epidemics seem to be related to specific meteorological factors (refs.). Smith and Jones (1995) have shown... Adams (1997) found that ... The correlations between these meteorological factors and subsequent malaria epidemics, however, have never been systematically investigated.

If such correlations do indeed exist, meteorological factors might be used to predict local epidemics. In this study we take a first step in developing a predicting model.'
At this point, the reader should have a good idea of the focus of the research. Now it is time to "zero in" on the specific research questions.

Key element 2: the specific research questions - the basis of credible science
'The purpose of this study was to answer the following questions. (1) What retrospective meteorological factors, and what combinations of factors, correlate significantly with the occurrence of subsequent malaria epidemics in Ethiopia? (2) To what extent do they explain the variance of occurrence of subsequent epidemics?'
Notice that the research questions are stated in terms of the variables that were measures or observed, in this case, meteorological factors (the independent variables) and occurrence of epidemics (dependent/outcome variable). Furthermore, the questions state the relationships sought between the variables: correlations and explanation of variance of the dependent variable. Such specific research questions tie the story together—they focus on credible science.

Key element 3: a description of the methods you used to answer your research questions.
This section will later become your Methods section. For the storyline, avoid details and make it understandable to the non-expert. Note that it is in past tense - factual information about what you did in this study.
'In a retrospective study, we collected meteorological data for 10 local areas in Ethiopia. The data included rainfall, temperature, sunshine, AAA, BBB, CCC, and DDD and... We also collected data concerning malaria epidemics for the same areas. This data covered the years 1963 to 2006. We developed a statistical model to determine correlations, and find factors and combinations of factors explaining the variance of epidemics. Using an independent subset of the data collected, we determined the predictive power of the model.'
Notice that in this section the authors report 2 types of information: (1) how they collected data, and (2) how they determined relationship between the variables.

Key element 4: the major findings
This will later become your Results section.
'We found that factors AAA, BBB, and CCC correlated significantly with subsequent epidemics in all 10 of the local areas studied. In 3 of the areas, the combination of CCC and DDD correlated significantly.'
Notice that in this section the authors report the relationships between the variables that they found. These are historical facts and, therefore, reported in past tense.

Key element 5: the answers to the research questions - your interpretation of the factual findings.
This will become the beginning of your Discussion section.
Notice that the answer to the research question uses exactly the same words used to state the question. And, notice that it is not a summary of results, but the authors' interpretation of the results about how the world IS and, therefore, stated in present tense. Of course, in a pilot study such as this, the authors cannot yet present definitive answers, and they indicate that with the words "suggest" and "may."
'The results of our study suggest that factors AAA, BBB, and CCC correlate significantly with subsequent malaria epidemics in Ethiopia. Furthermore, the combination of factors CCC and DDD may account for about XX% of variance in some areas. If we can generalize our findings to other areas, our model will have a predictive power of...'

Key element 6: the consequences of the answers—the value of your work.
This will become the Conclusion section and it relates directly back to the first key element, the original big health-related problem. A Conclusion is NOT a summary of results, but it describes how the study helps to solve the problem—it ties the end back to the beginning. And, it suggests a next step toward solving the problem - it gives direction to research.

'We conclude that local meteorological data can be used to predict malaria epidemics. Our statistical model, developed in this pilot study, has a predictive power of about 30%. Although this is certainly a first step toward predicting malaria epidemics, we would like to considerably increase the predictive power. We think that inclusion of groundwater level might increase the model's predictive power. This factor is, however, not available in the databases we used and will have to be determined by other means. Furthermore, our model still needs to be validated in other areas.'

The example as running text - a stand-alone story that focuses on the point and value of the research.

'Predicting Malaria Epidemics in Ethiopia'

Introduction

Malaria is still the number one killer of all infectious diseases. Most deaths could be prevented, however, if adequate medical facilities and medicines were available at the beginning of an epidemic. After an outbreak of malaria, getting adequate medical facilities and medicines to the local area can take many weeks. Obviously, this time is truly lost time and, for many victims, fatal. If, however, malaria epidemics could be predicted in local areas, medical facilities and medicine could be mobilized where they will be needed and, thereby, save many lives. Predicting where and when an epidemic can be expected is, however, currently not possible.
Malaria epidemics are known to be related to weather conditions. Previous research has shown that malaria epidemics seem to be related to specific meteorological factors. The correlations between these meteorological factors and subsequent malaria epidemics, however, have never been systematically investigated.
The purpose of this study was to answer the following questions. What retrospective meteorological factors, and what combinations of factors, correlate significantly with the occurrence of subsequent malaria epidemics in Ethiopia? To what extent do they explain the variance of occurrence of subsequent epidemics?

Methods

In a retrospective study, we collected meteorological data for 10 local areas in Ethiopia. The data included rainfall, temperature, sunshine, AAA, BBB, CCC, and DDD and... We also collected data concerning malaria epidemics for the same areas. This data covered the years 1963 to 2006. We developed a statistical model to determine correlations, and find factors and combinations of factors explaining the variance of epidemics. Using an independent subset of the data collected, we determined the predictive power of the model.

Results

We found that factors AAA, BBB, and CCC correlated significantly with subsequent epidemics in all 10 of the local areas studied. In 3 of the areas, the combination of CCC and DDD explained XX% of the variance in occurrence of subsequent epidemics.

Discussion

The results of our study suggest that factors AAA, BBB, and CCC correlate significantly with subsequent malaria epidemics in Ethiopia. Furthermore, the combination of factors CCC and DDD may account for about XX% of variance in some areas. If we can generalize our findings to other areas, our model will have a predictive power of about 30%.

Conclusion

We conclude that local meteorological data can be used to predict malaria epidemics. Our statistical model, developed in this pilot study, has a predictive power of about 30%. Although this is certainly a first step toward predicting malaria epidemics, we would like to considerably increase the predictive power. We think that inclusion of groundwater level might increase the model's predictive power. This factor is, however, not available in the databases we used and will have to be determined by other means. Furthermore, our model still needs to be validated in other areas.

As a running text, it is now a short (452 words) and understandable story that forms the skeleton for the journal article. All we need to do now is to fill in the scientific and technical details - without destroying the storyline. To ensure that your article clearly presents the point of your research, write a similar short storyline for your study. Then fill in the details (theory, references, methods, data, tables, figures etc.) needed to support that storyline.

Do not underestimate the difficulty of getting out of that box of technical details. The author of the above example was also in a box, a box full of complex statistical methods and computer algorithms to design his model. And, as a consequence, he had lost all sight of the health-related problem he was helping to solve - malaria. I hope this example will help you to get started.

- Ed Hull -

Advice to the reviewers of EJM

In the process of reviewing a paper, please refer to the following points

- Your first step should be to evaluate your relationship with the authors. To ensure the credibility of the process, reviewers should not have a conflict of interest with the authors. If this is a case, the paper should be appointed to other reviewers. Please keep us informed whether conflict of interest is an issue for you as an appointed reviewer.
- Is this work relevant and interesting for EJM?
- Are the objectives appropriate and clearly stated?
- Are the data valid?
- Are the conclusions valid and properly supported?
- Is the already existing work described adequately?
- Paper structure/organization; is this logical?
- Does abstract clearly convey meaning of the paper?
- Is the paper well written and can be easily understood? (Please keep in mind that students don't have the experience to reed throughout the paper very quickly and to understand everything in a research paper at the first glance)
- Are all sections really needed, or could they be shortened?
- Is the science reliable? Please, be aware of ethical issues such as plagiarism!

Comments should be detailed and specific. Mentoring the authors includes helping authors improve their paper under review even if these papers will/could not be accepted for publication in our journal. By careful reviewing, you will help improving the quality of papers published elsewhere too. Avoid vague complaints and provide appropriate citations if authors are unaware of the relevant work.

Please consider a manuscript received for reviewing as a confidential document and do not discuss the content of this paper with others. To maintain the validity of this process, you should never contact the authors about the paper under review.

The review process serves two important goals: providing guidance to the authors to improve the quality of their paper, and providing the editor or editorial board with valuable recommendations regarding the acceptance or rejection of the peer-reviewed papers (along the whole spectrum of major revision- minor revision- rejection). So it is important that you give comments to the authors, and to the editor in separate sections. Please use the provided form, because this makes life easier for you, the editor and the authors.

EJM is committed to rapid editorial decisions and publication. We request that reviewers return their comments within the time indicated at invitation. If any unanticipated difficulties arise that may prevent you from submitting the review on time, contact us by sending an email to the editor at erasmusjournalofmedicine@gmail.com, or the editorial office at ejm@erasmusmc.nl. You are welcome to contact us if you have any questions.

For more information about guidelines for the review process, please visit our website: www.erasmusmc.nl/ejm. We also recommend you to view the presentations of the EJM workshop on our website.
Here you can find instructions about how to scan through a paper and grab its essence, and how to structure your comments to the authors and to the editor.

Erasmus Journal of Medicine