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Brugada ECG pattern

Colophon

The Erasmus Journal of Medicine (EJM) is a scientific magazine by and for students, especially 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 is published on paper (1500 copies) and on the EJM website (www.erasmusjournalofmedicine.nl).

The main purpose of the EJM is to encourage medical and research master students to conduct research (empirical studies or systematic reviews) and report on this research, and become acquainted with a professional publishing process either as authors, reviewers or editors. A secondary purpose is to make the results of excellent student-driven research known to others.

The journal contains articles describing original research, systematic reviews, extended abstracts (summaries of recently conducted studies), calls from research projects for students to participate, opinion papers written by students, editorial comments, case reports, clinical lessons, clinical images, and letters to the editor.

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Foreword

Medical Delta: Pushing boundaries

Erasmus MC wants to provide the best possible healthcare and keep healthy people healthy as long as possible. We do this through groundbreaking research, education and training and by providing healthcare. All in accordance with the latest insights and the latest techniques.

The future of healthcare lies in convergence; in connections between medical disciplines and disciplines such as technology and information technology. Medical Delta, the consortium of Erasmus MC and Erasmus University Rotterdam with LUMC, Leiden University and TU Delft, anticipates on this development.

Medical Delta is a research-driven life sciences and medical technology cluster. Its institutes fuel many joint educational programs at BSc and PhD level in life sciences, biomechanical engineering, molecular sciences and entrepreneurship. For example, the Research Master in Clinical Technology, in which we provide professionals with medical and technical skills, so that they can develop innovative diagnostic and treatment methods and thereby make a difference to the health of the future.

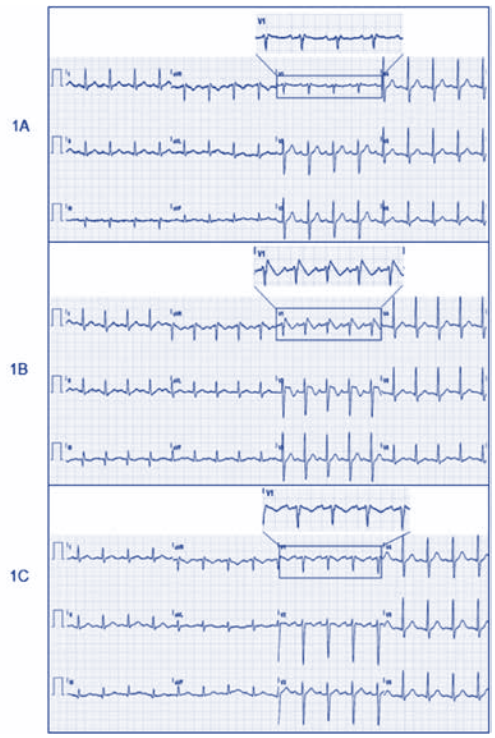
But Medical Delta also fuels many joint professorships. The first eleven Medical Delta professors were inaugurated on 12 June. This was unique in the Dutch academic history. Their goal: to improve healthcare using new technologies.

Physicians and engineers work together in the Delta Medical alliance, with each of them having a dual appointment at Erasmus MC and LUMC and TU Delft. For example, one professor develops special heart catheters that are inserted into the veins. A fast rotating motor sends light and sound into the veins so that very precise images of the veins can be made. This enables the targeted treatment of ailments before they lead to a heart attack or stroke. A fellow professor develops irradiation methods that reduce the likelihood that surrounding tissues are affected. A third colleague has devised a way of reducing the need for revision surgery after knee or hip replacement. Instead of the patient having to undergo surgery again, we inject quick drying cement between the prosthesis and the bone using hollow needles.

Technology plays an important role in providing early diagnosis, improving minimally invasive treatment methods and furthering the development of personalized medicine - making it easier to tailor medical treatment to the characteristics of each individual patient. In the Medical Delta alliance we sustainably improve healthcare, among others, by applying the enormous advantages of medical technology. Our goal: to provide the best possible healthcare and keep healthy people healthy as long as possible.

Professor Jaap Verweij
Dean and Vice-chairman Erasmus MC Board of Directors

Erasmus Journal of Medicine



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EJM: facing new challenges

In its 4th year of publication, Erasmus Journal of Medicine is an established scientific journal of medical faculty born to promote the conduct of scientific research by medical students, and to train research-oriented doctors. In the light of the newest developments in the filed of Medical Delta, the editorial board has come to realize that this is the moment to move forward and to push the boundaries of educational goals in order to fulfill with a broader extent of scientific needs.

Organized participation of joint educational programs at BSc and PhD level in life sciences, biomechanical engineering and molecular sciences compose new challenges we are facing now. Greater access for all Medical Delta students and specially for an extended spectrum of Erasmus University students encompassing not only the preclinical medical students but also the research masters, the clinical internships and fellows will help to work on these challenges. The Research Masters in Clinical Technology and Nanobiology aiming to train professionals with medical and technical skills will be provided with the possibilities to mutually communicate with medical students through this scientific plathform.

The presented journal is the product of the hard work delivered by many. Each submitted paper has been peer-reviewed after the first triage by students and staff members of the editorial board: Ron de Bruin, Paul van Daele, Tom Birkenhäger and our student-editors Erik Dieters, Sid Morsink, Mostafa Mohseni, Iris van der Sar, Geertje de Boer, Fatih Incekara and Sandrine Nugteren. Our student team has been renewed with new members who will be responsible for the next issue. Without the valuable work of our editorial assistant Petra Erkens this achievement would have never been possible. Proudly we present an issue with a variable content including papers in the following sections: opinion papers, systematic reviews, extended abstracts, and case reports. We hope that also this issue of the journal will stimulate students to write and submit their work to us. I invite you to read the journal's seventh edition and to help us improving it by providing us with your feedback: ejm@erasmusmc.nl.

These new challenges we are facing now present new opportunities as well: opportunities to grow, to improve, and to learn new skills and abilities, which we might not learn otherwise.

Ajda T. Rowshani, MD, PhD
Internist (Chair of the editorial board)
Rotterdam, June 2014

Foreword

At the Erasmus MC, a wide variety of education is offered. Not only doctors-to-be, but also students from the five different research masters, nanobiology and soon clinical technology join together at our medical faculty. In addition, a lot of PhD-students are conducting science at the Erasmus MC. The EJM is aimed at all of those. We want to motivate you to become involved in research early in your careers. Our journal offers the possibility to get introduced to the publication process and learn about how to do a peer-review.

In parallel, we aim to broaden the range of articles in the EJM to the full scope of research: from preclinical to clinical studies. From fundamental laboratory studies to case reports, it's all interesting for our readers. Students from all different disciplines are important providers of these articles. In this way, we can learn from each other's knowledge and expand our own range of interests. For research master students, the EJM is a great way to present their work after performing an internship. PhD-students are given the opportunity to send their extended abstracts and overviews.

In the meantime, we are also working on getting the EJM registered in PubMed. So in the future, it might be possible for you to reach an enormous audience by publishing in our journal.

In this issue, you can read up on the treatment options in antibody-mediated rejection after kidney transplantation and deepen you knowledge of TRALI, the most serious cause of transfusion morbidity and mortality. Furthermore, critical insights are given on placebo and the doctor patient relationship and we learn about the pros and cons of solarium use under the age of 18 years. Of course, there's more to find out in this issue.

We hope you enjoy reading this edition of EJM and we look forward to your contributions!

Student editors of EJM

Geertje de Boer
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Mostafa Mohseni
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A rational approach to antibody mediated rejection

The systematic review by Jharap et al. on the treatment of antibody mediated rejection addresses one of the key problems of modern kidney transplantation.[1] The development of the C4d staining 20 years ago and the subsequent analyses of transplant biopsies have made clear that B-cell mediated rejection is of utmost importance in kidney transplantation. C4d is seen as a footprint of immunoglobulin mediated immunity but has limited sensitivity and in the case of blood group incompatible transplantation also has limited specificity. The development of high sensitivity assays for the detection of antibodies directed against donor HLA has proven to be of great additional importance in the detection of antibody mediated rejection. In fact, the latest version of the BANFF guideline on the diagnosis of rejection allows for the diagnosis of humoral rejection in the absence of a positive C4 d staining in the presence of renal histology compatible with antibody mediated rejection in combination with the detection of donor specific antibodies.[2]

However, while the entity of humoral rejection has been defined clearly and its importance in transplantation outcome is undisputed little is known about the optimal treatment. This review gives a highly welcome overview in which the sparse literature largely consisting of case series is analyzed in a concise fashion. Only one study in this series is a true randomized controlled trial on the treatment of antibody mediated rejection.[3] This trial studied the effect of immunoadsorption compared with no specific therapy directed against the humoral component. Unfortunately this trial was stopped early after including only 5 patients per treatment arm due to a very poor outcome in the control arm.

The published case series use various regimes consisting of intravenous immunoglobulins either alone or with plasma exchange, rituximab or anti T-cell treatment in various combinations. Most of these series do not have a control group. Due to the poor quality of the published trials it is difficult to draw firm conclusions. Possibly the only statement that can be made with reasonable confidence is that treatment for ABR can help.

This review helps to identify a number of highly relevant questions. While it is clear that plasma filtration or immunoadsorption have a beneficial effect is not clear what the contribution of adding IVIG to this treatment is.

This means that we have a very weak scientific basis for the current gold standard of treatment consisting of plasma exchange in combination with IVIG. It is noteworthy that a number of studies additionally use T-cell depleting treatment. The frequent presence of T-cells in the infiltrate in humoral rejection give a rationale for this approach. However, the contribution of additional T-cell depletion to the effectiveness and risk of rejection treatment in this setting is totally unclear.

The Jharap et al. acknowledge this lack of high quality data and suggest a trial in which standard treatment with plasma exchange and IVIG is compared with the complement inhibitor Eculizumab. Alternatively, treatment with proteasome inhibitors that target plasma cells is a logical approach that needs proper evaluation in randomized controlled trials.[4] It is highly unfortunate that the transplant community has not succeeded in establishing a soundly evaluated standard of treatment with which these new treatment modalities can be compared. Hopefully the transplant community will cooperate to make these important trials possible.

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Educate the mind, protect the skin

In the current issue of the EJM Marije Notenboom argues that solarium use should be prohibited for people under the age of 18. With the ever-increasing rate of skin cancer worldwide and also in the Netherlands, this could be a way to try to stop this trend.

The Scientific Commission on Consumer Products of the European Commission states that tanning devices should not be used by individuals under the age of 18 years. The minimum age of using tanning studios, set by the Food and Consumer Product Safety Authority (Nederlandse Voedsel- en Warenautoriteit), is 18 years old but this is not legally binding.

Fact is that UV-radiation poses a major problem in terms of health. In a globalizing society where the costs of transportation have decreased significantly more people are enjoying their vacations in sunny resorts. With this in mind, a prohibition of solarium use might not have a great impact on skin cancer incidence. Children should be warned against the negative effects of excessive sun-exposure like the carcinogenic properties of UV-radiation and the premature ageing of the skin. They should be aware of the precautionary measures they can take to minimise sun damage to their skin.
Knowledge is power, and people should be informed about UV-radiation risks at a young age in the same way children are informed about sexually transmitted diseases. It should be incorporated in the curriculum of high schools, and both teachers and doctors should actively participate in educating our youth about these risks.

This could trigger a change in culture where a tanned skin is no longer regarded as a sign of living an active healthy lifestyle, but rather as taking an irresponsible risk to develop skin cancer.

A prohibition of solarium use for people under the age of 18 is definitely a step forward, but the first step to reduce the growing incidence of skin cancers would be to advocate the implementation of an educational program at early age.

On behalf of the student board of the EJM

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Systematic review

The anti-inflammatory effects of fluoxetine on activated microglia *A systematic review*

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Summery

Introduction: **This article reviews current literature about the anti-inflammatory effects of Fluoxetine on activated microglia in the brain. Evidence of this effect would strengthen the belief that inflammation plays a role in the development of depression and explain the therapeutic effect of SSRI's.**
Methods: **A search in PubMed for the Medical Subject Headings (Mesh) term Microglia in combination with the Mesh term Fluoxetine or Serotonin Uptake Inhibitors.**
Results: **We found 7 eligible articles. Fluoxetine treated microglia showed significant reduction of microglia activation in 2 articles and 4 articles showed significant reduction in nitric oxide (NO) and inducible nitric oxide synthase (iNOS). Inflammatory-markers were also significantly reduced in 5 articles and 4 articles showed significant suppression of NF-ϰB activity. Reactive oxygen species (ROS) were significantly reduced in 3 articles. 6 studies found a reduction of micoglial activity and 4 found significant reductions.**
Conclusion: **In all studies we found at least 2 significant reductions in microglial inflammatory parameters due to Fluoxetine. All articles showed reduction in inflammatory parameters. This supports the idea that SRRI's have an anti-inflammatory effect in the brain.**

Introduction

Selective Serotonin Re-uptake inhibitors (SSRI's) are a group of anti-depressants, often prescribed for depression because they are safe and well tolerated.[1-3] The effect of SSRI's used to be explained by the monoamine theory which stated that depression was caused by a deficiency of serotonin. However, as proven with tryptophan depletion experiments, this could not explain the entire effect of the SSRI's.[4,5]

In recent years there is the increasing amount of evidence for the role of the immune system in the pathogenesis of depression. Studies have proven that the activity of the innate immune system is increased in depression, usually with elevated plasma levels of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6]) and C-reactive protein (CRP). [6-10] Because of this speculation arose that SSRI's may partially work through an anti-inflammatory effect.[11-13] It was for example found that Fluoxetine, a SSRI, suppressed the number of inflammatory cells and the TNF-α release from monocytes in animal models.[14]

The question is however, if this anti-inflammatory effect translates to the brain. This finding would strengthen the belief that inflammation plays a role in the development of depression and explain the therapeutic effect of SSRI's. This article investigates if current literature supports the anti-inflammatory effect of SSRI's in the brain.

The central component in the inflammatory reaction in the brain is the microglia, a macrophage specific for the central nerve system.[15] In activated state these microglia produce various pro-inflammatory cytokines, such as TNF-α, and no longer function in a growth stimulating way as they do in steady state.[16]

This article reviews current literature about the effects of Fluoxetine, a commonly prescribed SSRI, on activated microglia to examine its anti-inflammatory effect in the brain.[3] Microglial activation was achieved by treatment with lipopolysaccharide (LPS), kainic acid (KA), 1-methyl-4-phenyl-pyridinium (MPP+) or by causing cerebral ischemia in several animal models.[17-23]

Methods

We conducted a search in PubMed at January 26th 2013. We searched PubMed for the Medical Subject Headings (Mesh) term Microglia in combination with the Mesh term Serotonin Uptake Inhibitors or Fluoxetine. Our exact search was: "Microglia"[Mesh] AND ("Serotonin Uptake Inhibitors"[Mesh] OR "Fluoxetine"[Mesh]). Based on title and abstract we included all articles which studied the effects of Fluoxetine on activated Microglia. We only looked at the response of activated microglia to Fluoxetine to prevent confounding factors. No other in- or exclusion criteria were necessary. All studies compared Fluoxetine treatment with either a placebo or no treatment in activated microglia.

We looked at the effects of Fluoxetine on microglial activation in general and more specifically several parameters which show inflammation, namely production of nitric oxide (NO), inducible nitric oxide synthase (iNOS), pro-inflammatory of nitric oxide (NO), inducible nitric oxide synthase (iNOS), pro-inflammatory markers, reactive oxygen species (ROS) and transcription factor Nf-kB.

Results

Our PubMed search produced 14 articles. After applying the inclusion criteria, 7 articles remained [17-23], which investigated the effect of fluoxetine on activated microglia. All articles were published within the last 4 years, as seen in Table 1. Microglia were cultured from rat brain [18,20,23], mouse brain [19,21] and BV2 microglial cell lines [17,22]. The mircoglia were activated using LPS, MPP+, KA or cerebral ischemia. Six studies investigated the microglia separately [17,18,20-23], one study measured microglial parameters within hippocampus regions. All articles defined statistical significance as $p < 0.05$.

Dosage

Out of the 7 studies, 5 used different dosages of Fluoxetine. [17,19,20,22,23] In these 5 studies, we noticed in at least one microglial parameter, that a lower dose yielded no significant reduction whereas a higher dose did. One study mentioned a significant pro-inflammatory effect on activated microglia which were treated with a lower dose of Fluoxetine.[17]

In Table 2 we gathered data regarding the highest doses of Fluoxetine administered, and our following results are also based on this.

Microglia activation

Except for one study, all studies researched the effect of Fluoxetine on microglia activation.[17-23] In order to do this the 6 studies used different markers (Table 1). All 6 studies found reduction in microglia activation by Fluoxetine treatment (Table 2). Two of these studies mentioned a significant reduction [20,23], whereas the other 4 only visualised the results using images of the microglia markers, without p-values.[18,19,21,22]

Microglial inflammatory markers

Different techniques were used to measure the effect of Fluoxetine on microglial inflammatory markers (Table 1). One study measured inflammatory marker protein expression, which was found to be significantly reduced in Fluoxetine treated microglia.[17] Two studies measured RNA expression of inflammatory markers, one of which observed a significant reduction in Fluoxetine treated microglia.[19,20] Three studies measured both RNA and protein expression of inflammatory markers.[21-23] Both RNA and protein expression of inflammatory markers were significantly reduced in Fluoxetine treated microglia in all 3 studies.[21-23]

Table 1 - Study Characteristics

Article	Mechanism of microglia activation	Microglia source	Parameters investigated
Tynan et al, 2012 [17]	LPS	BV2 microglial cell line	TNF-α, NO
Chung et al, 2010 [18]	LPS	rat brain (SN)	activation (ED1, OX-42), iNOS, NADPH oxidase, ROS, protein carbonyl
Jin et al, 2009 [19]	KA	CA1 and CA3 mouse hip-pocampus regions	activation (Iba-1), COX-2#, IL-1β#, TNFα#, NF-κB (IκBα)
Lim et al, 2009 [20]	LPS, cerebral ischemia	rat brain, primary microglia culture	activation (Iba-1, Mac2), COX-2#, IL-1β#, TNFα#, iNOS#, NO, NF-κB
Chung et al, 2011 [21]	MPP+	mouse brain (SN), microglia culture	activation (ED1, Mac1), IL-1β*, TNFα*, iNOS*, NO, NADPH oxidase, ROS, protein carbonyl
Liu et al, 2011 [22]	LPS	BV2 microglial cell line, primary microglial culture	activation (CD11b), TNF-α*, IL-6*, iNOS*, NO, NF-κB (IκBα, p65, DNA binding)
Zhang et al, 2012 [23]	LPS, MPP+	rat brain cultures	activation (Iba-1), TNF-α*, IL-1β*, iNOS#, NO, ROS, NF-κB (p65, IKKb, IκBα)

RNA expression was studied

* RNAand protein expression were studied

Abbreviations: LPS; lipopolysaccharide, SN; substantia nigra, KA; kainic acid, MPP+; 1-methyl-4-phenylpyridinium, TNF-α; tumour necrosis factor-α, NO; nitric oxide, iNOS; inducible nitric oxide synthase, IL; interleukin, ROS; reactive oxygen species, NF-κB; nuclear factor-kappaB, IκBα; nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha, IKKb; inhibitor of nuclear factor kappa-B kinase subunit beta, COX; Cyclooxygenase, NADPH; Nicotinamide adenine dinucleotide phosphate.

Table 2 - Effects of the highest Fluoxetine dose on activated microglia

Article	activation	NO	iNOS	pro-inflammatory factors[a]	NF-κB	ROS
Tynan et al, 2012 [17]		↓		↓		
Chung et al, 2010 [18]	↓*		↓			↓
Jin et al, 2009[b] [19]	↓*			↓	↓	
Lim et al, 2009 [20]	↓	↓	↓*	↓*	↓	
Chung et al, 2011 [21]	↓*	↓*	↓	↓		↓
Liu et al, 2011 [22]	↓*	↓	↓	↓	↓	
Zhang et al, 2012 [23]	↓	↓	↓	↓	↓	↓

Empty boxes represent parameters that were not determined in the concerning study
[a] Any of the following molecules: COX-2, TNF-α, IL-1β, IL-6
[b] Studied the effects on NO and NF-kB in the whole hippocampus
↓ significant reduction, $p < 0.05$
↓* Visualised reduction without p-value

Oxidative stress

Three studies assessed the influence of Fluoxetine on ROS produced by activated microglia.[18,21,23] In all 3 studies ROS was significantly lower in Fluoxetine treated microglia (Table 2). Furthermore, 5 studies examined NO production by activated microglia, 4 of which mentioned significant reduction in Fluoxetine treated microglia (Table 2).[17,20-23] The expression of iNOS in Fluoxetine treated microglia was also measured in 5 articles using iNOS mRNA expression, protein levels, or both.[18,20-23] Three studies found a significant reduction in the mRNA expression of iNOS.[21-23] Two studies found a significant reduction in the iNOS protein expression.[18,21] More reductions in iNOS mRNA and protein expression were found, unfortunately these results were not quantified, but only visualised.[18,20,22]

NF-κB activity

The NF-κB pathway activity was studied in 4 articles.[19,20,22,23] All 4 studies found that the NF-κB activity was significantly supressed in Fluoxetine treated microglia.

Discussion/Conclusions

For a long time the effect of SSRI's was explained by the inhibition of serotonin re-uptake.[1-5] However, in the last years, increasing evidence has been found that this group of anti-depressants also exerts an anti-inflammatory effect.[14] It is important to study if this effect translates to the brain because it would give a new perspective on the development and treatment of depression. It also suggests that SSRI's could be used in the treatment of inflammation.
Therefore in this review recent literature was studied to give a current view on the anti-inflammatory effect of SSRI's in the brain.

We limited ourselves to the effect of Fluoxetine on activated Microglia because SSRI's seem to have an inflammatory effect in steady state microglia.[24] To eliminate unknown differences in effects between SSRI's we chose to look only at Fluoxetine, one of the most studied SSRI's and different dosages, at its highest dosage.

We found significant and visualised reductions due to Fluoxetine in microglial activation and inflammatory factors in all studied disease models, both in vivo and in vitro.

At least 2 of the following parameters were significantly reduced in all studies: microglial activation, NO, iNOS, ROS, and Nf-kB production, and pro-inflammatory marker expression (COX-2, TNF-α, IL-1β, IL-6). The effect seems to be dose-dependent in most studies. Six studies looked at microglial activation. All found a reduction of activity and 4 found significant reductions. This makes it likely that fluoxetine does have an anti-inflammatory effect in the brain.

The anti-inflammatory effect in the brain can also help to explain the functional pathway of SSRI's in depression. The underlying mechanism of this anti-inflammatory effect however is still unknown. One hypothesis for the mechanism of action is the suppression of NF-κB by Fluoxetine.[14] NF-κB is an important transcription factor for the production of pro-inflammatory factors like TNF-α, NO and IL-6 via the transcription of Nos2.[17,24] However, the effect seems strongly dose and cell dependant and it is not known how Fluoxetine exactly supresses Nf-kB.[20,24]. This mechanism should be further investigated. Possibly, this knowledge could lead to a better understanding of the pathogenisis and succesful treatment of depression.

This orientating review only studied the effect of the highest dosage of SSRI's. We also limited ourselves to Fluoxetine to prevent confounding factors because of possible differences between SSRI's. In the orientation process these were necessary but important limitations but we encourage future research to compare all types of SSRI's at different dosages.

Also, some of the studies only visualised effects of Fluoxetine without quantifying these differences or calculating if these differences were statistically significant. This makes it difficult to compare findings. We advise future researcher to quantify their research data instead of solely using visualisation, and to give clear explanation about the dosage used.
Despite the limitations of our review, all research supports the anti-inflammatory effect of Fluoxetine on the brain. This could lead to new insights in the pathophysiology of depression and development of new, more effective treatments.

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Risk factors of transfusion related acute lung injury

A systematic review

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Summery

Objective: **The aim of this study was to determine the risk factors of Transfusion-Related Acute Lung Injury (TRALI).**
Methods: **Pubmed was searched; original studies, with a control group, about individual risk factors of TRALI in adults, were included.**
Results: **Seven studies met the selection criteria, and were consequently included. Ultimately 24 risk factors were assessed, 2 of which (amount of plasma and amount of platelet units transfused) were unanimously proven to be significant. Leukoreduced PRBCs, RBCs from female donors, age of RBC units, anti HNA, male sex, smoking, diabetes, CABG and aspiration were proven to not influence the risk of TRALI. All other risk factors remained controversial in these studies. After correction for multiple testing, only one of the risk factors (amount of platelet units transfused) remained significant in one study.**
Conclusions: **We can conclude that many of the risk factors for TRALI remain controversial.**

Introduction

Transfusion- related acute lung injury (TRALI) is the most serious cause of transfusion related morbidity and mortality.[1-4]

The two-hit theory is generally accepted for TRALI pathogenesis.[5] The first hit involves neutrophil sequestration and priming in the lung microvasculature. This is due to recipient factors such as endothelial injury. 'Priming' refers to the neutrophils shifting to a state in which they respond to an otherwise weak signal.[6] The second step is activation of recipient neutrophils, this happens through a factor in the transfused blood product. Associated with activation is the release of cytokines, reactive oxygen species, oxidases, and proteases from neutrophils. These damage the pulmonary capillary endothelium, which leads to pulmonary edema.

The characteristic clinical presentation of TRALI is the sudden onset of hypoxemic respiratory insufficiency during or shortly after the transfusion of a blood product. [3,7] Symptoms may be delayed as long as six hours, but usually begin within one to two hours of the initiation of the blood component infusion.[3] The most common signs and symptoms of TRALI are as follows.[8]

- Hypoxemia: in an intubated patient this could manifest as a change in oxygenation or increased oxygen requirements (100 percent, by definition)
- Bilaterally pulmonary infiltrates on chest radiography (100 percent, by definition)
- If previously intubated, pink secretions from the endotracheal tube (56 percent)
- Fever (33 percent)
- Hypotension (32 percent)
- Cyanosis (25 percent)

Incidence reports on TRALI vary widely, ranging from 0.08% to 8% per transfused patient.[9-10] Although the absence of specific disease markers and diagnostic tests has resulted in this large variation of incidence, TRALI is generally considered being a rare event.

While there might be a global trend of increased awareness for TRALI amongst clinicians and researchers[11], TRALI is still underdiagnosed and underreported.[12-14] Cases remain unnoticed or are misdiagnosed as acute lung injury (ALI) or fluid overload of other etiology.[12,14,15] Knowledge of risk factors may help to identify patients at risk for TRALI; in this way it will be possible for doctors to properly assess the risks and benefits of a blood transfusion. Furthermore, knowledge about risk factors may influence the selection criteria for blood donors and thus limit the number of TRALI cases.

To investigate the risk factors for TRALI, a systematic review was performed. All publications on PubMed were analysed to determine what factors have been found to significantly influence the risk of developing TRALI.

Methods

PubMed was used as a medium to search for articles. Several Mesh-terms were used to make our search as comprehensive as possible. The following search protocol was used: "Acute Lung Injury "[MAJR] AND "Blood Transfusion/adverse effects"[MAJR] AND ("Risk"[Mesh] OR "incidence"[Mesh]) AND "humans"[MeSH Terms] AND (loprovinleurlib[SB] OR "loattrfree full text"[sb])

Inclusion and exclusion criteria
The search was conducted by 2 independent reviewers, disagreements were resolved by a third reviewer. Articles had to be written in English and be published between January 1, 1995 and January 9, 2013. Furthermore, articles had to be available in the Erasmus MC library. Following the initial search, titles and abstracts were checked for relevance.

The main topic had to be the finding of risk factors for TRALI. Articles were excluded if they were not about risk factors. Only articles based on randomized controlled trials, retrospective studies, comparative studies, case-control studies and controlled clinical trials were included. Systematic reviews, reviews, case reports and editorials were excluded.

The references of the remaining articles were checked to search for more other relevant studies.

Data analysis
The articles were analysed in order to produce an overview of all the included articles with author, date, journal, study type, number of subjects enrolled, country and method of data collection.

Statistical analyses
Some of the included articles did not give p-values, but medians, odds ratios and hazard ratios. In those cases the p-values were calculated in order to be able to determine the significance (alpha level = 0.05). Furthermore the Bonferroni correction method was used if the risk factors were significant.

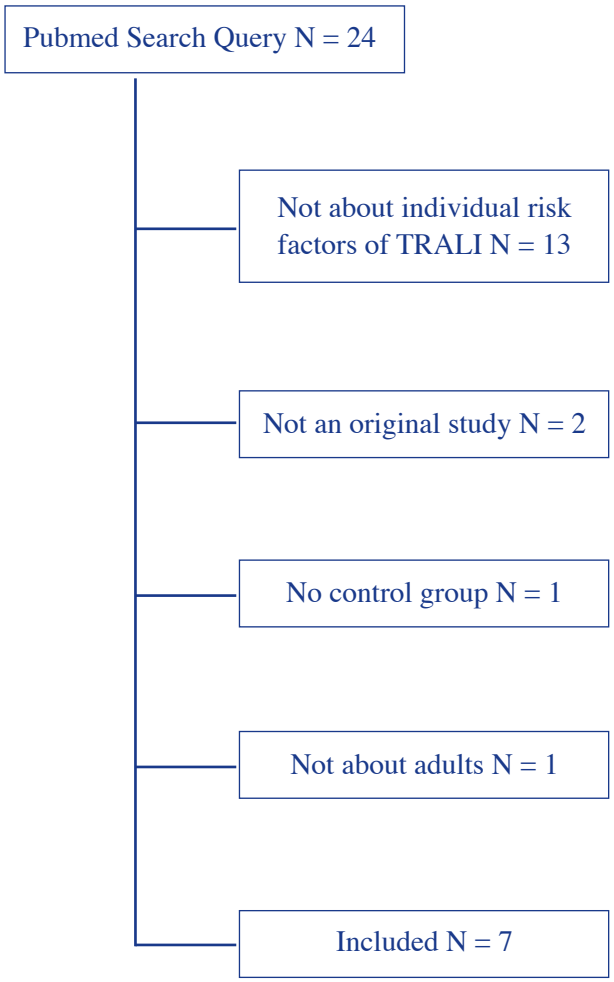
The Bonferroni correction concerns the question if, in the case of doing more than one test in a particular study, the alpha level should be adjusted downward to consider chance capitalization. This is the case when in a single study more than one hypothesis is evaluated, each hypothesis with a single test. Because the alpha level of each test was set at 0.05, at least one in twenty of the hypothesis tested would be significant, due to chance fluctuation.
The Bonferroni correction was applied for all the significant results, the p-value was divided by the number of tests done in the article.

Results

Study Selection
Our PubMed search produced 24 articles; based on title and available abstract a total of 7 articles met the inclusion criteria and were selected for full article review.[16-22] Reference checking did not produce extra articles. A flowchart illustrating the selection of articles in each stage of the systematic review is presented in figure 1. From these articles, all risk factors that were mentioned in at least 2 articles were included.

Study Characteristics
A summary of study characteristics of included articles can be found in Table 1. All included articles were English language, case-control studies or retrospective cohort studies, published between 2010 and 2012. Sample sizes of included studies ranged from 48 to 525.

Figure 1- Article selection



Blood components received
The amount of platelets and plasma units transfused where significant risk factors, while the amount of leukoreduced PRBCs transfused was not.
Whether the amount of RBC units, nonleukoreduced PRBC units and FFP units transfused are risk factors, turned out to be uncertain. [table 2] Specific confidence intervals were not provided, due to the lack of data in the majority of original articles.

While receiving RBCs from a female donor was clearly not influencing the risk of TRALI, receiving plasma from a female donor remained an unclear factor. The age of RBC units was not a risk factor, neither was the age of platelets. [table 2]

Antibodies in blood units
Anti HNA was not found to be a significant factor. Whether Anti-HLA Class I and/or II are risk factors for TRALI remained undetermined. [table 2]

Table 1 - Study Characteristics

Author	Journal	Study type	No. subjects enrolled	Country	Data collection
Benson AB (2010 Oct)	Intensive Care Med	Case-control	225	University of Colorado Hospital (UCH) – Colorado, US	Patients with primary diagnosis of GI-bleeding who were admitted to the medical ICU from 01/2002 through 07/2008
Benson AB (2011 Feb)	Liver Transpl	Retrospective cohort study	525	University of Colorado hospital (UCH) – Colorado, US	Identified patients who underwent liver transplantation between 2002 and 2009 at the university of Colorado
Middelburg RA (2012 Mar)	Transfusion	Case-control	60	Netherlands	All TRALI patients reported in the Netherlands from 01/2005- 12/2007
Middelburg RA (2010 Nov)	Transfusion	Case-referent study	83	Colorado, Netherlands, poland, finland, Spain, UK, Minnesota (US)	International, multicenter, data collection between 06/1991 – 10/2007
Toy P (2012 Feb)	Blood	Case-control	253	California (US), Minnesota (US)	Enrollment at 2 tertiary care medical centers (the University of California-San Fransisco and the Mayo Clinic) from 03/2006-12/2009
Vlaar AP (2010 Mar)	Crit Care Med	Nested case-control study	114	Netherlands	All patients admitted to the ICU of a university hospital were screened for onset of TRALI, from 11/2004 through 10/2007
Vlaapr AP (2011 Apr)	Blood	Nested case-control study	48	University of Amsterdam, Netherlands	Screened 1000 cardiac surgery patients from 11/2006 through 2/2009

Table 2 - Descriptive data of components received and antibodies in blood units

Risk factor	No. studies	Significant factor (after correction for multiple testing)*	Not a significant factor
Amount of transfusion			
Number of RBC units	5	Benson AB 2010 Oct (n) Toy P 2012 Feb (n) Vlaar AP 2011 Apr (n)	Benson AB 2011 Feb Vlaar AP 2010 Mar
Nonleukoreduced PRBC's	2	Benson AB 2010 Oct (-)	Benson AB 2011 Feb
Leukoreduced PRBC's	2		Benson AB 2010 Oct
Number of (apheresis) platelet units	4	Benson AB 2010 Oct (n) Toy P 2012 Feb (y) Vlaar AP 2010 Mar (n) Vlaar AP 2011 Apr (n)	Benson AB 2011 Feb
Number of plasma units	3	Toy P 2012 Feb (n) Vlaar AP 2010 Mar (n) Vlaar AP 2011 Apr (n)	
Amount of units FFP transfused	3	Benson AB 2010 Oct (-) Vlaar AP 2011 Apr (n)	Vlaar AP 2010 Mar
Transfusions from female donors			
Plasma from female donor	3	Toy P 2012 Feb (n)	Vlaar AP 2010 Mar
RBCs from female donor	2		Middelburg RA 2010 Nov Toy P 2012 Feb
Age of transfusion products			
Age of RBC units	4		Middelburg RA 2012 Mar Toy P 2012 Feb Vlaar AP 2010 Mar Vlaar AP 2011 Apr
Age of platelets	4		Middelburg RA 2012 Mar (n) Toy P 2012 Feb Vlaar AP 2010 Mar Vlaar AP 2011 Apr
Antibodies in blood units			
Anti HNA by GIFT	2		Vlaar AP 2011 Apr Toy P 2012 Feb
Anti-HLA-Class I	2	Vlaar AP 2011 Apr (n)	Toy P 2012 Feb
Anti-HLA-Class II	2	Vlaar AP 2011 Apr (n)	Toy P 2012 Feb

*"y": yes, "n": no and "-" :the articles did not give the specific p-values (e.g.: P=<0.01)

Table 3 - Characteristics of patients

Risk factor	No. studies	Significant factor (after correction for multiple testing)*	Not a significant factor
Age	5	Vlaar AP 2011 Apr (n)	Benson AB 2010 Oct
			Benson AB 2011 Feb,
			Toy P 2012 Feb
			Vlaar AP 2010 Mar
Male	4		Benson AB 2011 Feb
			Toy P 2012 Feb
			Vlaar AP 2010 Mar
			Vlaar AP 2011 Apr
Smoking	3		Toy P 2012 Feb
			Vlaar AP 2010 Mar
			Vlaar AP 2011 Apr
Chronic alcohol abuse	3	Toy P 2012 Feb (n)	Vlaar AP 2010 Mar
			Vlaar AP 2011 Apr
APACHE II	2	Vlaar AP 2010 Mar (n)	Benson AB 2010 Oct
Diabetes	2		Vlaar AP 2010 Mar
			Vlaar AP 2011 Apr
CABG	2		Vlaar AP 2010 Mar
			Vlaar AP 2011 Apr
Sepsis	2	Vlaar AP 2010 Mar (n)	Benson AB 2010 Oct
Aspiration	2		Benson AB 2010 Oct
			Vlaar AP 2010 Mar
Serum albumin g/dl	2	Benson AB 2010 Oct (-)	Benson AB 2011 Feb
MELD score	2	Benson AB 2010 Oct (-)	Benson AB 2011 Feb

*"y": yes, "n": no and "-" :the articles did not give the specific p-values (e.g.: P=<0.01)

Characteristics of patients

Of the two demographical characteristics researched in the included articles, only male sex was unanimously deemed not significant. Whether age is a risk factor remained controversial, as one study found it to be significant, while four others did not. [table 3]

Neither, smoking, diabetes, CABG or aspiration were found to be significant risk factors. Whether chronic alcohol abuse, APACHE II, sepsis, serum albumin levels or MELD score are risk factors for TRALI remains to be determined. [table 3]

Bonferroni correction

After correction for multiple testing, using the Bonferroni method, all results turned out to be not significant except for one study. The one exception is the amount of platelet units transfused (p: 0.00235, the Bonferroni correction threshold was p<0.00357).

A substantial amount of articles could not be corrected for multiple testing, because the p-values were not given (e.g.: P<0.01) and the p-value could not be calculated because the required data was not available. An overview of risk factor significance is shown in table 4.

Table 4 - Overview of risk factors

Risk factor	No. studies	Significant risk factor for TRALI (after correction for multiple testing)*
Amount of transfusion		
Number of RBC units	5	Unclear (no)
Nonleukoreduced PRBC's	2	Unclear
Leukoreduced PRBC's	2	No
Number of (apheresis)	4	Yes (unclear)
platelet units		
Number of plasma units	3	Yes (no)
Amount of units FFP	3	Unclear
transfused		
Transfusions from female donors		
Plasma from female donor	3	Unclear
RBCs from female donor	2	No
Age of transfusion products		
Age of RBC units	4	No
Age of platelets	4	Unclear (no)
Antibodies in blood units		
Anti HNA by GIFT	2	No
Anti-HLA-Class I	2	Unclear (no)
Anti-HLA-Class II	2	Yes (no)
Age	5	Unclear (no)
Male	4	No
Smoking	3	No
Chronic alcohol abuse	3	Unclear (no)
APACHE II	2	Unclear (no)
Diabetes	2	No
CABG	2	No
Sepsis	2	Unclear (no)
Aspiration	2	No
Serum albumin g/dl	2	Unclear
MELD score	2	Unclear

Discussion/Conclusions

The aim this this review was to assess the risk factors for TRALI. After searching PubMed and references for relevant articles, 7 studies were included. From these articles, each risk factor that was mentioned in at least 2 articles was included.

Risk factors for TRALI are not consistently proven significant. There was not a single significant risk factor unanimously proven to influence the risk of TRALI after adjusting for multiple testing. Before multiple testing, only the number of platelets and plasma units transfused were found to be significant risk factors for TRALI in all studies. Leuko-reduced PRBCs, RBCs from female donors, age of RBC units, anti HNA, male sex, smoking, diabetes, CABG and aspiration were proven to not significantly influence the risk of TRALI. All other risk factors remained controversial in these studies. This study shows that a lot of work is still to be done. Many of the risk factors of TRALI remain controversial. The risk factors that were unanimously proven to not influence the risk of TRALI should still be a topic of research. When more research becomes available concerning a presumed unsignificant risk factor, the evidence will become stronger. Also, renewed research methods and scientific breakthroughs can radically change conventional views oncertain risk factors.

It would be interesting to study these risk factors in specific studies. Clearly, running 16 tests for different risk factors in one study only amounts to a threshold p-value that is too low ($P<0.003$) to let anything turn out to be significant.

Limitations

One limitation of this study is the small number of articles in our meta-analysis. However, the total number of subjects is large and we believe that the total sample size is large enough to draw meaningful conclusions. A second possible limitation is that the largest studies were performed in the United States of America, which could lead to a rather 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 severe measure of multiple testing. Several authors reject this method because it significantly increases the chance of a type II error. Less severe methods may result in more proven risk factors. However, in this review, the importance of avoiding a type I error, was considered more important than the avoiding of type II errors.

In conclusion, our study provides evidence that many of the risk factors for TRALI remain controversial.

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Tyrosine kinase inhibitors in the treatment of systemic sclerosis *A systematic review*

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Abstract

Objective: **The objective of this systematic review is to determine the efficacy and safety of tyrosine kinase inhibitors (TKIs) in the treatment of systemic sclerosis.**
Methods: **A systematic literature search for clinical trials that describe the efficacy of TKIs in the treatment of systemic sclerosis (SSc) was performed in MEDLINE. Our primary outcome measures included forced vital capacity (FVC%), modified Rodnan skin thickness score (MRSS) and diffusion capacity for carbon monoxide (DLCO). A quality assessment for the included studies was performed.**
***Results*: Five clinical trials have been included, in which a total of 79 patients were treated with imatinib and 14 patients with placebo therapy. Two clinical trials reported significant improvement in skin disease and one showed FVC% improvement.**
***Conclusion*: The studies did not provide enough evidence to conclude that the treatment with imatinib benefits all patients with SSc. The total dropout rate because of imatinib-related adverse events was 20%.**

Introduction

Systemic sclerosis (SSc) is a multisystem autoimmune disease characterized by immune cell activation, vasculopathy and excessive fibrosis in various tissues.[1] Current treatment options are limited and include immunosuppressive therapies like methotrexate and mycophenolic acid. However, the mortality rate is approximately five- to eightfold higher than that of the general population, when adjusted for age and gender.[2] Pulmonary fibrosis and/or pulmonary hypertension are the main causes of mortality in patients with SSc.[2-4]

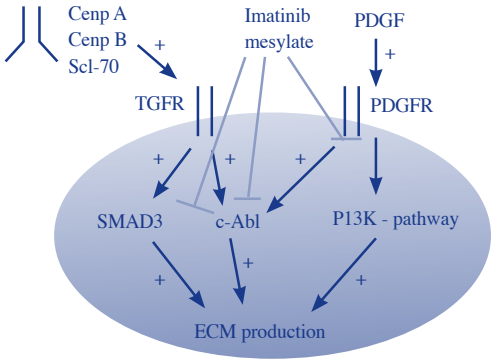
In the pathogenesis of systemic sclerosis transforming growth factor beta (TGFβ), Smad, Abelson kinase (c-abl) and platelet-derived growth factor receptor (PDGFR) play a crucial role.[1,5-8] In serum of SSc patients increased levels of fibroblast-stimulating autoantibodies such as cenpA, cenpB, scl-70 and platelet-derived growth factor (PDGF) are found when compared to healthy controls.[7]

Imatinib mesylate (Gleevec) was initially developed for the treatment of patients with chronic myeloid leukemia (CML). In CML imatinib mesylate inhibits the activity of the fusion protein Bcr-Abl, which is the main cause of disease in CML.[9] Imatinib mesylate is a tyrosine kinase inhibitor (TKI) that blocks the activity of c-abl, Smad1 and PDGF, hereby inhibiting the fibrotic response.[8]

An important feature in the development of fibrotic diseases is the overstimulation of the PDGFR and TGFβ-receptor pathways in fibroblasts that induce synthesis of extracellular matrix (ECM). Stimulation of TGFβ-receptors contributes to production of various types of collagen, fibronectin and actin.[8] Both PDGFR and TGFβ-receptor are transmembrane receptors that, after stimulation, form intracellular dimers through phosphorylation. These dimers activate protein complexes such as smad4 for TGFβ-receptor and the PI3K-pathway for PDGFR. Smad proteins are intracellular proteins that are able to act as transcriptional factors via binding with the promoter region of a gene.[8]

Imatinib mesylate treatment results in dual inhibition of the PDGF and the TGFβ signalling pathway by blocking the downstream mediators smad3 and c-abl of TGFβ and by blocking PDGF.[10] This is illustrated in figure 1.
Imatinib mesylate has shown to be effective in murine and in *in vitro* models of fibrosis and was found to reduce collagen production.[10]

Figure 1- The effect of imatinib mesylate on a fibroblast



Imatinib mesylate blocks PDGF and the downstream mediators smad3 and c-abl of the TGFβ pathway. This results in a decrease of ECM production (collagen, fibronectin, actin).

The first case-report of a patient with SSc responding to treatment with imatinib mesylate was published in 2008.[11] Subsequently, case-reports and the first clinical trials followed. However, definitive evidence of the efficacy and safety of imatinib mesylate has not been delivered. Therefore, it remains unclear what the current role of imatinib mesylate is in the treatment of SSc.

The main objective of this systematic review is to analyse all clinical trials that describe the efficacy and safety of tyrosine kinase inhibitors in the treatment of SSc.

Methods

Search strategy

A systematic literature search for relevant studies was conducted on 9 January 2014 in Medline/Pubmed. The following search criteria were used: "Scleroderma, Systemic"[Mesh] AND "Protein Kinase Inhibitors"[Mesh] AND Clinical trial[ptyp].

Only articles written in English were included. Additionally, our research was limited to human subjects. Our last search was performed on 27 January 2014.

Criteria for studies included in this review

Exclusively clinical trials that described the efficacy of TKIs in the treatment of SSc were included. There were no limitations in outcome measures used.

Study selection

We separately screened all abstracts and individually assessed full-text articles for eligibility. The articles were assessed according to the criteria mentioned under "criteria for studies included in this review".

Data extraction

We analysed the outcome measures forced vital capacity (FVC%), modified Rodnan skin thickness score (MRSS) and diffusion lung capacity for carbon monoxide (DLCO) to compare the efficacy of imatinib mesylate on the skin and lungs reported in the included studies. The FVC%, MRSS and DLCO are commonly used parameters to define the severity of SSc. When the outcome parameters reported significant improvement, the treatment was considered effective.

Figure 2- Quality assessment (QA)

Was the study described as randomized?	1/0
Was the study described as double blind?	1/0
Was there a description of withdrawals and dropouts?	1/0
Did the study include a control group?	1/0
Did the study include more than 10 patients?	1/0

The following questions were asked when reading the clinical trials. When the answer is positive for a study, the study receives one point. The higher the score, the higher the quality of the article is.

We analysed data about adverse events (AEs) when reported. We evaluated the number of patients who discontinued the trials to assess the impact of the adverse events. Also, we reported the number of AEs and severe adverse events (SAE) and described these.

Quality assessment

We performed a quality assessment (QA) in order to base our conclusions on the most reliable articles, as assessed by objective criteria. The quality assessment is based on the JADAD score. Moreover, we added two criteria as presented in figure 2. The quality assessment consists of five criteria which all include factors that improve the power of evidence of the study. The higher the score, the higher the quality of the included study. In general the included studies have a small study population, which might have biased the presented results. Therefore we added one criterion that takes into account the size of the study population. Finally, the presence or absence of a control group was included as a criterion.

Results

Overview of study characteristics

Our systematic literature search resulted in five relevant studies.[12-16] After application of the inclusion criteria these five articles remained. The characteristics of the studies are presented in table 1.

These studies together included 79 patients who were treated with imatinib mesylate and 14 patients on placebo therapy.

All studies used the same TKI: imatinib mesylate, although different doses were used. In the study by Khanna et al.[13] patients started at 100 mg/day and doses were increased with 100 mg every two weeks to a maximum dose of 600 mg/day. The mean ± SD dosage used was 445 ± 125 mg/day. The median dosage used was 400 mg/day. In the studies by Pope et al.[12] and Sabnani et al.[15] a dose of 200 mg/day was prescribed. In the studies performed by Prey et al.[14]
and Spiera et al.[16] all patients started with a dose of 400 mg/day. However, in the study by Prey et al. at the end of the study the dose varied from 100-400 mg/day per patient. Patients were allowed to continue baseline therapy consisting of immunosuppressive agents, such as cyclophosphamide (CYC) in three studies.[12,14,15]

Four studies had a follow-up duration of twelve months.[13-16] The study of Pope et al. had a follow-up duration of six months.[12]

Various endpoints were described to measure the clinical improvement of SSc. Four studies used DLCO [13-16], three studies used FVC% [13,15,16] and four studies used MRSS.[12-14,16]

Overview of study results: efficacy

An overview of the study results on efficacy is presented in table 2.

A one-year phase I/II, open-label pilot trial performed by Khanna et al. reported an increase in FVC% and DCLO (respectively 1,74% and 1,46%). However, this increase was not significant (P>0.05).[13]

Table 1 - Study characteristics

Author	QA*	Population (n)	Mean age of patients (years)	Gender (Female)	Dose of imatinib mesylate used	Duration of follow-up (months)	Outcome measures	Co-medication used
Khanna et al. [13]	2	20	46.1	65%	600 mg/day (started at 100 mg/day and increased 100 mg every 2 weeks)	12	DLCO** FVC*** MRSS**** TLC*****	No
Pope et al. [12]	3	10	51 (40-67)	70%	200 mg/day	6	DLCO FVC MRSS TLC	Methotrexate
Prey et al. [14]	5	28	48.9 (30-71)	61%	400 mg/day	12	DLCO MRSS	Immunosuppressive medication, bosentan, calcium, channel blokkers, corticosteroid (<20mg)
Sabnani et al. [15]	1	5	50 (36-62)	40%	200 mg/day (100-400 mg/day)	12	DLCO FVC TLC	Cyclophosphamide
Spiera et al. [16]	2	30	Median 48 (18-71)	80%	400 mg/day	12	DLCO FVC MRSS	No

* QA: Quality assessment score
** DLCO: Diffusion capacity for carbon monoxide
*** FVC: Forced vital capacity
**** MRSS: Modified Rodnan skin thickness score
***** TLC: Total lung capacity

Table 1 presents the study characteristics, including: QA, number of patients included, patient characteristics, dose of imatinib mesylate used, the duration of follow-up and the outcome measures used.

Table 2 - Efficacy and adverse events reported

Author	FVC% after follow-up	MRSS after follow-up	DLCO after follow-up	Number of patients discontinued due to AE/SAE	AE**	SAE***
Khanna et al. [13]	+ 1,74% (P>0.05)	Improved 3.9 units (p<0.001)	Improved 1,46% (p>0.05)	7 out of 20	5 out of 20 patients	3 out of 20 patients
Pope et al. [12]	ND*	Improved 0.7 units (p>0.05)	ND	5 out of10	In 7 out of 10 patients	ND
Prey et al. [14]	ND	No significant difference between placebo and imatinib	0.00 (-0.10 - 0.07) (p=0.1964)	1 out of 28	53 (imatinib) with respect to 39 (placebo)	1 patient
Sabnani et al. [15]	+ 80-89% of predicted in one patient	ND	same patient 43-50% of predicted	3 out of 5	3 patients	0
Spiera et al. [16]	Improved from a mean of 82.9±21.1% to 89.3±25.2% predicted (p=0.008)	Improved 6.6 points (95% CI −4.5 to −8.7)	Improved from 78.0 ± 22.9% to 83.5 ±29.2% (p=0.12)	0 out of 30	total 358 AE	Total of 24 SAE. 1 SAE related to imatinib

* ND : Not defined, the clinical trial did not include this outcome.
** AE : Adverse event(s)
*** SAE : Severe adverse event(s)

Table 2 presents the results of efficacy of all clinical trials included. The number of AEs and the number of patients discontinued due to AE/SAE reported are also shown. FVC, MRSS, DLCO are outcome measures for efficacy.

Spiera et al. conducted a one-year phase IIa, single-arm, open-label clinical trial.[16] In this study, FVC% significantly improved from a mean of 82.9±21.1% to 89.3±25.2% predicted (p=0.008) and the DCLO improved insignificantly from 78.0 ± 22.9% to 83.5 ±29.2% (p=0.12).

Furthermore, Spiera and colleagues found that patients without interstitial lung disease showed significantly better results for treatment with imatinib mesylate than patients with interstitial lung disease (ILD).[16] In Spiera et al. patients without ILD improved on average 10.4% point with respect to their FVC% (-3.3 to 18.2; p=0.01). Patients with ILD remained stable with an average increase of 2.1% point.[16]

The phase II double-blinded randomized controlled trial performed by Prey et al.[14] did not demonstrate any significant increase in FVC% or DCLO.

Sabnani et al. included five patients with restrictive lung disease in their study.[15] Four of these patients had severe restrictive lung disease and one had mild restrictive lung disease. Only the patient with mild restrictive lung disease showed improvement. This patient improved 80-89% in FVC% and 43-50% of predicted in DCLO.[15]

Pope et al. performed a six-month, randomized, double blind, placebo-controlled pilot study, which did not measure lung function parameters but only parameters regarding cutaneous SSc.[12] The results regarding skin sclerosis will be mentioned further on.

Almost all studies reviewed the effect of imatinib mesylate on skin sclerosis. The MRSS improved in the study by Khanna et al.[13] with 3.9 points (P<0.001). Spiera et al.[16] found a 6.6-point increase (95%CI -4.5 to -8.7). Both Pope et al.[12] and Prey et al.[14] did not report any significant difference in MRSS after follow-up.

Although there are some positive results in reduction of skin involvement, the studies with the most power of evidence based on our quality assessment did not report any success. [12,14] Previous studies suggested the use of imatinib mesylate in lower dosages in order to minimalize adverse events while maintaining the reduction of skin involvement.[[17] The studies included in this review did not confirm these findings.

Overview of study results: safety

In our review 20% of the patients discontinued the study because of AEs.

In terms of treatment-related AEs, the following was found. Three studies reported patients who discontinued the study due to AEs or SAEs.[12,13,15]

Khanna et al. reported seven patients who discontinued the study, of which five patients discontinued because of AEs and three patients because of SAEs. One of these patients had both AEs and SAEs. Of the five patients reported with AEs two were also reported to have discontinued the study because of the underlying SSc. Three patients were found to develop SAEs. Of these SAEs, two were related to the underlying disease. The other SAE patient stopped the imatinib mesylate treatment because of dyspnea and generalized edema that was resistant to treatment with diuretics.[13]

In the study of Pope et al., five out of ten patients discontinued the study because of AEs. These AEs included fluid retention, weakness, nausea, vomiting and chest pain. Pope et al. study was stopped because even after reintroduction of medication of lower dosage a lack of tolerability remained. [12]

All patients tolerated the combination therapy of imatinib mesylate and CYC in the study of Sabnani et al.[15] Only one patient had periods of required intermittent discontinuation because of fluid retention due to imatinib mesylate.
In the study by Prey et al. two patients discontinued treatment as result of an AE. One of these patients terminated participation because of SAEs (anasarca) and the other temporarily quit treatment because of AEs (edema).[14]

In contrast, Spiera et al. found one imatinib-related SAE, which was fluid overload with bilateral pleural effusions. [16] Common AEs, which required dose adjustment, were musculoskeletal complaints, fluid retention, gastrointestinal complaints, intercurrent illness and constitutional symptoms.

The most frequently reported adverse events were edema and gastro-intestinal complaints.[12-14,16] Both were very likely found as result of imatinib mesylate usage. Khanna et al.[13] reported a general rash in a patient. This rash disappeared after terminating imatinib mesylate therapy, and reappeared when the therapy was resumed.

Tachyarrhythmia/cardiomyopathy is a serious AE described in patients treated with imatinib mesylate for CML. In the studies we included this SAE was reported in one patient of the study of Spiera.[16]

An overview of the study results on AE-related study discontinuation is also presented in table 2.

Discussion

Despite the promising results of preclinical studies and case reports, substantial benefit of imatinib mesylate in the treatment of SSc has not been proven by the clinical trials included.

One weakness of this review is the inclusion of mostly open-label clinical trials. This is due to the fact that placebo-controlled trials are difficult to perform for a rare disease like SSc. Conclusions about efficacy and safety are uncertain due to lack of a control group.

Firstly, spontaneous improvements in skin scores are seen in patients with early stage diffuse cutaneous SSc[18], which emphasizes the need for randomized controlled trials. Most autoimmune diseases know periods of exacerbation and remission and this phenomenon might explain the fluctuation in MRSS rather than effective treatment.

Furthermore, the study population included patients with heterogeneous underlying syndromes and different phases of SSc. This probably has effects on the outcome of the efficacy of therapy in SSc. This was shown in the study of Sabnani et al.[15] where only the patient with mild restrictive lung disease showed improvement. In further research, defining specific groups could lead to more definitive conclusions.

In addition, the patients included have had different treatment for SSc before and during imatinib mesylate therapy. It was shown by The Scleroderma Lung Study in 2009 that previous use of immunosuppressive agents like CYC can affect the efficacy outcome of the studies.[19]

The clinical significance of improvement of lung disease in relation to imatinib mesylate is hard to interpret because of the variability between patients. Not all subjects with SSc-associated interstitial/restrictive lung disease have been administered the same dose of imatinib mesylate. We expect that progressive lung involvement will eventually result in fibrosis, which is not reversible in its end-stage. In the early stages of SSc, the inflammation and fibroblast activation are more pronounced and the treatment has more benefit. In the end-stage of fibrosis, fibroblasts possibly exert their effects via more paracrine and autocrine pathways. For this reason medication may have less benefit in end-stage SSc.

Many of the AEs described are most likely related to the underlying SSc rather than imatinib mesylate. Prey et al. showed the same AEs in the imatinib mesylate group as in the placebo group, for example infections and haematotoxicity. [14] The studies showed heterogeneity in AEs, which could be related to heterogeneity of SSc. Future cohort studies should give more definitive conclusions about AEs of imatinib mesylate in the treatment of SSc patients.

Finally, most patients treated with imatinib mesylate did not respond to regular treatment. It is possible that patients that have not responded to previous treatment modalities are less likely to respond to imatinib mesylate. However, we tried to minimize these limitations of the review by performing a QA and include all published clinical trials until now.

Clinical trials that were randomized, double blinded, described the withdrawals and dropouts, included a control group and/or included more than ten patients contribute to minimizing the limiting items mentioned above.

Quality assessment

The study of Prey et al.[14] was found to be the clinical trial with the highest quality. Prey et al.[14] has a QA of 5 (randomized, double-blind, description of withdrawals and dropouts, control group, study population of more than 10 patients). The QA of Pope et al.[12] was 3 (randomized, double blind, placebo-controlled). The QAs of Khanna et al.[13]and Spiera et al.[16] were 2.

Sabnani et al.[15] has the lowest QA with a QA of 1 (description of withdrawals and dropouts).

The low number of patients included should be taken into account because false conclusions can be drawn. Studies with a low total number of patients included are less likely to find a significant result and deliver poor level of evidence.

This suggests that studies with the highest QA are also most likely to present the most reliable result, which in this case is also not in favour of treating SSc patients with imatinib mesylate. Prey et al.[14] did not find any significant difference in all three outcome measures.

Conclusion

Even when taking into account the limitations of the included clinical trials, we can conclude the following.

Firstly, two out of five studies showed significant improvement in MRSS [13,16], one out of five studies showed significant improvement in FVC%.[16] These studies do not provide enough evidence to conclude that the treatment with imatinib mesylate offers benefit for all patients with SSc, especially when taking into account the QA for these studies. However, the case reports of Tamaki et al.[17] and Van Daele et al.[11] showed that imatinib mesylate had a promising effect for cutaneous involvement. Future randomized controlled trials should provide clarity about the effect of imatinib mesylate for cutaneous involvement.

Second, the AE of imatinib mesylate in the treatment of SSc with the highest incidence is edema, followed by gastro-intestinal discomfort. Many studies reported adverse events and had a high rate of withdrawals or dropouts because of AEs. However, many of the AEs described are very possibly correlated to the underlying SSc and not (fully) caused by imatinib mesylate. In our review 20% of the patients discontinued the study because of AEs directly related to imatinib mesylate. Considering the severity of SSc this stands in proportion.

We recommend performing prospective randomized trials with larger study populations in the future. Due to the fact that SSc has a heterogeneous presentation, these trials should differentiate between the presentation and phase of SSc in the patients included. Groups treated with imatinib mesylate should be compared to a group treated with imatinib mesylate plus immunosuppressive agents, a group treated with only immunosuppressive agents and a placebo-controlled group.

Until further larger studies are performed we consider the use of imatinib mesylate therapy in individual SSc patients with skin involvement when regular treatment options have failed.

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Efficacy and safety of TPO-receptor agonists in chronic immune thrombocytopenic purpura (ITP) in adults *A meta-analysis*

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Objective: **To determine the efficacy and safety of two TPO receptor agonists (TPO-RAs) in the treatment of chronic ITP in adult patients. We compared these drugs to each other, placebo and the standard of care.**
***Methods*: We searched PubMed in January 2013 for eligible randomized controlled trials (RCTs). The primary outcome was the platelet response and the secondary outcomes were bleeding episodes and other adverse events. A meta-analysis was performed to assess the effect of TPO-RAs on platelet response.**
***Results*: We selected 7 studies, which included 845 patients in total (592 TPO-RA and 253 either placebo or standard of care). The response rates were significantly higher for TPO-RAs compared to placebo (RR 3.83; 95 CI 2.78 – 5.29; p<0.001). TPO-RAs decreased bleeding episodes in all studies. Generally, TPO-RAs were well tolerated; grade 3-4 adverse occurred in only 3% to 15% of patients.**
***Discussion*: TPO-RAs are an effective and safe treatment for chronic ITP in adults. However, as platelet counts return to baseline after discontinuation of treatment, further research is needed to assess the efficacy and safety of long-term use.**

Introduction

Immune thrombocytopenia (ITP) is an acquired autoimmune disorder that is characterized by thrombocytopenia (platelet count < 150 x 10^9/L) due to accelerated clearance and destruction of antibody-coated platelets by tissue macrophages, predominantly in the spleen. Antiplatelet antibodies also target antigens on megakaryocytes, suppressing platelet production.[1]

ITP in adults generally appears in a chronic form, which is defined variously as at least 6 months duration to at least 12 months duration.[1] Symptoms are variable, ranging from mild bruising or mucosal bleeding to florid hemorrhage.
Most serious bleeding occurs in patients with platelet counts below 10 x 10^9[2] A platelet count lower than 30 x 10^9/L and/or bleeding symptoms are an indication for treatment of patients with ITP. Treatment is usually not necessary for patients with platelet counts > 30 x 10^9/L, without bleeding symptoms, trauma or surgery.[3]

For patients with chronic ITP, treatment with corticosteroids (prednison) is the standard first-line therapy. After initial treatment with glucocorticoids, most patients require second-line medical treatment or splenectomy.[3] The frequency of long term complete remission after a course of first-line therapy with corticosteroids ranges from 10%-30%.[4] This means that after initial treatment with prednisone, most patients require second-line therapy. There are several therapeutic options, such as splenectomy, rituximab and trombopoietin-receptor agonists (TPO-RAs).[1]

Splenectomy is the most frequent used second-line therapeutic option in patients who do not respond to prednison. However, splenectomy has several disadvantages. The response to splenectomy cannot be predicted. Two thirds of the patients who have undergone splenectomy require additional therapy.[5] The mortality and complication rates of laparoscopic splenectomy are 0.2% en 9.6% respectively.[6] Splenectomy may increase morbidity from venous thromboembolism. Splenectomized patients have an increased risk of developing sepsis caused by encapsulated bacteria and thus require antibiotic prophylaxis.[7,8]

Treatments aimed at increasing thrombopoiesis, such as the thrombopoietin receptor agonists (TPO-RAs), may be more effective and safe in the second-line therapy of ITP. Two TPO-RAs are currently approved for use in ITP: romiplostim and eltrombopag.[1] Romiplostim and eltrombopag bind to the TPO-receptor on megakaryocytes and stimulate megakaryocyte proliferation and differentiation into thrombocytes.[9] Although studies suggest that treatment with TPO-RAs increased the platelet count[4,10] it remains unclear which treatment is most effective and safe as second-line therapy of ITP and what the place is of TPO-RAs in this therapy.

Therefore, in a meta-analysis of the literature of adult patients with chronic ITP, we addressed the following research questions. Are romiplostin and eltrombopag effective at increasing platelet counts relative to placebo and the current standard of care? If so, which treatment is more effective? Are romiplostin and eltrombopag effective at decreasing the risk of bleeding episodes?

Methods

Literature search

We searched PubMed on January 10, 2013, using keywords related to immune trombocytopenic purpura and TPO-receptor agonists. The exact search strategy included the following keywords: ("Purpura, Thrombocytopenic, Idiopathic "[Mesh] OR ITP[tiab] OR chronic immune thrombocytopenia[tiab] OR idiopathic thrombocytopenic purpura[tiab]) AND ("Receptors,Thrombopoietin/agonists"[Mesh] OR Romiplostim[tiab] OR Eltrombopag[tiab]) AND ("clinical trial"[ptyp] OR "randomized controlled trial"[ptyp]) AND English[lang].

Inclusion criteria

We included trials which met the following criteria in the title or abstract: 1. the trial studied adult patients (male or female aged ≥ 18 years) with chronic immune trombocytopenic purpura; 2. the study design was a randomized controlled trial; 3. the trial reported one or more of the following outcome measures: platelet response, bleeding symptoms and adverse events/safety. We included only randomized controlled trials to make a reliable comparison of TPO-RAs versus placebo.

Using these inclusion criteria, we independently evaluated the eligibility of the studies we found in PubMed.

Outcome measures

The primary outcome was the platelet response, defined as > 50 x 10^9/L and at least doubling of the baseline values. The secondary outcomes were bleeding episodes and adverse events.

Data extraction

We extracted the relevant information from the selected studies. The data collection included the following study characteristics: study design, country, duration, sample size, median age of the participants, selection criteria, type of intervention and the reported outcome measures.

If two or more studies presented the same data from the same trial, we included those data only once in our analysis.

Quality assessment

We evaluated the methodological quality of the included studies using the Delphi list by Verhagen et al.[11]

The criteria for assessing the quality of the individual RCTs were as follows: performance of a method of randomization, concealed treatment allocation, group similarity at baseline, specified eligibility criteria, blinding of the outcome assessor, care provider and patient, presentation of point estimates and measures of variability and intention to treat analysis.

One point was granted if the criterion was fulfilled; if there was uncertainty about whether the given criterion was fulfilled, zero points were given. If it was certain that the criterion was not fulfilled, 1 point was subtracted. We added funding by the pharmaceutical industry as an extra criterion, for which we also subtracted 1 point. We classified the trials according to the total points they scored. Trials with ≥ 5 points were qualified as high quality studies, 3 or 4 points corresponded with medium quality and ≤ 2 points with poor methodological quality.

Main and subgroup analyses

The main analysis was the effect of TPO-receptor agonists overall (both the romiplostim group and the eltrombopag group) on the platelet count in patients with chronic ITP. We performed a subgroup analysis for both romiplostim and eltrombopag to assess the platelet response.

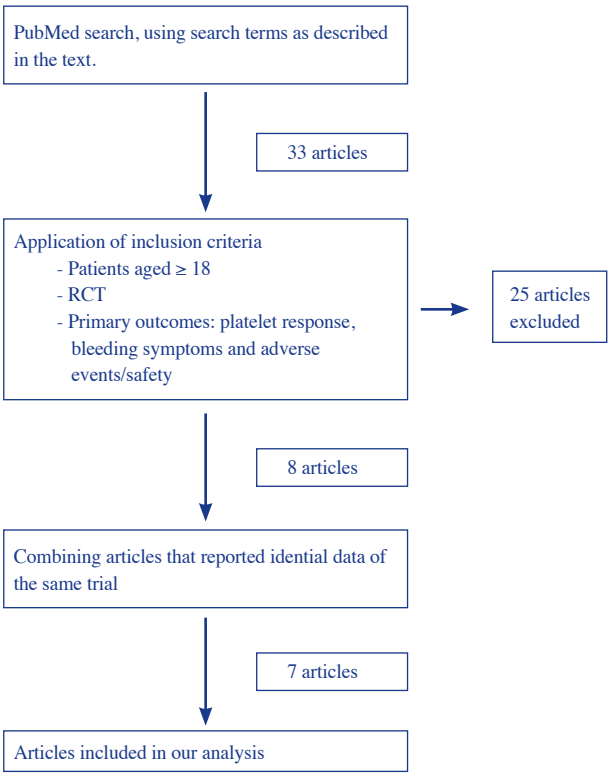
Statistical analysis

We determined the effect of TPO-RAs on platelet response using meta-analysis. We first determined heterogeneity of the included studies by calculating I2, which quantifies the percentage of variation attributable to heterogeneity. [12] I2 was calculated with the Q Cochrane heterogeneity statistic. We considered an I2 value < 50% to be acceptable. We pooled relative risk (RR, 95% CI) using fixed effects model. Comprehensive Meta-analysis 2.2.064 software was used for the statistical analyses.

Results

Our Pubmed search produced 33 articles, from which eight articles were selected by applying the inclusion criteria described above. After reading the full text we identified two articles (Gernsheimer and Kuter) that reported on the same study. Those were taken together, in accordance with our review protocol (see Figure 1)

Figure 1- Flowchart of the selection process



Study characteristics

Of the seven studies that were included, four focused on eltrombopag and two on romiplostim. In total, these studies included 845 patients, of which 592 received a TPO-RA and 253 either placebo or standard of care. Two studies [13,14] were conducted in Japan, the others were multicenter studies that included patients from Europe, the USA, Australia, New Zealand and East Asia. The median age of participants ranged from 48 to 60 years. Stasi et al. included only patients with baseline platelet counts <50 x 10^9/L [15], while the other studies included patients with baseline platelet counts <30 x 10^9/L. All but one compared TPO-RAs with placebo; Stasi et al. compared with standard of care. Patients in this group were assigned treatments by the investigators based on the therapeutic guidelines that were valid at the time the study was conducted.[15] Table 1 shows the main characteristics of the studies and patients.

Quality assessment

We assessed the quality of all included studies; these results are shown in Table 1. Three studies [14,16,17] made clear statements on how allocation was concealed, whereas the others did not. The studies on eltrombopag [16,18,19] were sponsored by GlaxoSmithKline, the pharmaceutical company that manufactures eltrombopag, and the studies on romiplostim [14,15,17] were sponsored by AmGen Inc., the pharmaceutical company that manufactures romiplostim.

Table 1 - Characteristics of included studies

Study	Study Design, Country and Duration	Patients (no.; median age, selection criteria)	Intervention and control	Outcome Measures *	Quality
Cheng et al. (2011)	RCT; Europe, East-Asia, New-Zealand, USA; Nov 2006 – July 2007	197; 48 (SD or range not stated); platelet count <30 x 10^9/L, received previous treatment for ITP, at least a 6 month history	Standard of care** + 50 mg eltrombopag once daily vs. placebo	Platelet response***; bleeding symptoms, adverse events	High
Bussel et al (2009)	RCT; Australia, Europe, East-Asia, USA; Feb – April 2006	114, 48 (SD 17); platelet count <30 x 10^9/L, received previous treatment for ITP, at least a 6 month history	Standard of care + 50 mg eltrombopag once daily vs. placebo	Platelet response***, safety, tolerability, signs of bleeding	High
Bussel et al. (2007)	RCT; Australia, Europe, East-Asia, USA; Feb – Nov 2005	118; 50 (range 18-85); platelet count <30 x 10^9/L, received previous treatment for ITP, at least a 6 month history	30, 50 or 75 mg eltrombopag once daily vs. placebo	Platelet response***	Poor
Tomiyama et al. (2011)	RCT; Japan; Sep 2007 – June 2008	23; 60 (range 26-72); platelet count <30 x 10^9/L, received previous treatment for ITP, at least a 6 month history of ITP, Japanese race	12,5 mg eltrombopag once daily (adjusted to max. 50 mg) vs. placebo	Platelet response**, bleeding episodes	Moderate
Gernsheimer et al. (2008)	RCT; Europe, USA; Mar 2005 – Dec 2006	125;52 (range 21-88); platelet count <30 x 10^9/L, no active malignancy or history of stem cell disorder	1 µg/kg (adjusted to max. 10 µg/kg) romiplostim once weekly vs. placebo	Platelet response***, adverse events	High
Shirasugi et al. (2011)	RCT; Japan; Nov 2007 – April 2009	34; 55 (SD 13); platelet counts <30 x10^9/L, received previous treatment for ITP, at least a 6 month history , had H pylori eradication if proven H pylori positive, Japanese race	3 µg/kg (adjusted to max. 10 µg/kg) romiplostim once weekly vs. placebo	Platelet response***, bleeding symptoms, adverse events	High
Stasi et al. (2011)	Post hoc analysis of a RCT; Australia, Europe, USA; Dec 2006 – Sep 2007	234;57 (range 18-90); platelet counts <50 x10^9/L. received previous treatment for ITP, but no splenectomy.	3 µg/kg (adjusted to max. 10 µg/kg) romiplostim once weekly vs. standard of care	Bleedings related episodes	Poor

* Only the outcome measures considered to be of interest for this review.
** Treatments were selected by the investigators based on therapeutic guidelines.
*** Platelet response is defined as a platelet count > 50 x 109/L and at least twice as much as at baseline.

Platelet Response

Data on overall platelet responses were available for four studies on eltrombopag [13,16,18,19] and two on romiplostim.[14,17] The response rates were significantly higher for TPO agonists compared to placebo (RR 3.83; 95 CI 2.78 – 5.29; p<0.001) (figure 2). We found an RR of responding to eltrombopag of 3.32 (95% CI 2.36 – 4.67; p<0.001).

For romiplostim we found an RR of responding of 11.59 (95% CI 4.50 – 29.85; p<0.001).

Bussel et al. compared the efficacy of three doses of eltrombopag (30, 50 and 75 mg).[18] They found that only dosages of 50 or 75 once daily were effective; 30 mg once daily elicited a platelet response in 28% of patients, which was similar to the results achieved with placebo (11%; p=0.13). However, Tomiyama et al. reported platelet responses in 60% of patients (RD 60%, 95% CI 35.2-84.8%), with a mean dosage of 33.7 mg (range 12.5-50 mg) once daily.[13] Three studies investigated possible differences in treatment efficacy between splenectomized and non-splenectomized individuals.[16,18,19] No such difference was found.

For romiplostim, Gernsheimer et al. reported higher and more durable platelet responses in non-splenectomized individuals [17], but Shirasugi et al. did not find any difference.[14]

After discontinuation of treatment, platelet responses returned to baseline or close to baseline in all studies.[13,14,16-19]

Bleeding Episodes

All seven studies that assessed rates of bleeding showed that TPO-RAs decrease bleeding in patients with chronic ITP. This decrease was correlated with an increase in platelet count in all studies. Cheng et al. and Bussel et al. calculated the odds of bleeding during treatment (compared to placebo) and found Odds Ratios of respectively 0.24 (95% CI 0.16-0.38; p<0.0001) and 0.27 (95% CI 0.09–0.88; p=0·029).[16,19] Bleeding was measured using the WHO Bleeding Scale.[20]

Bussel et. al. mentioned decreased bleeding episodes in patients treated with 50 or 75 mg eltrombopag, but no exact data or statistical analysis were provided.[18] Tomiyama et al. reported a decrease in the proportion of patients with bleeding episodes from 48% at baseline to 5% at 6 months (no statistical analysis provided here either).[13]

Gernsheimer et al. did not find any difference in bleeding events (WHO Bleeding Scale Grade 1-4) between patients treated with romiplostim and patients treated with placebo (57% vs. 61%, p=0.68).

However, a significantly smaller percentage of patients in the romiplostim group had experienced clinically significant bleeding events (WHO Bleeding Scale Grade 2-4) than patients in the placebo group (15% vs. 34%, p=0.018).[17]

Shirasugi et al. reported a decrease of bleeding events from 42% (first 24 weeks) to 20% (week 96), but no data on comparison with placebo or statistical analysis was provided.[14]

Stasi et al. compared bleeding rates between patients treated with romiplostim and patients receiving standard of care (SOC). They found a relative rate of 0.33 (95% CI 0,27 – 0,40) favouring romiplostim.[15]

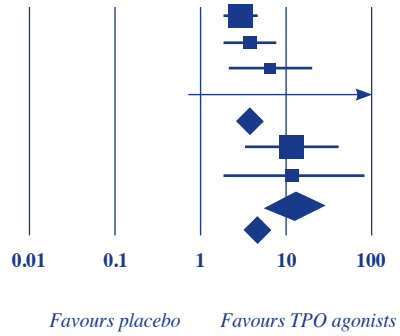
Adverse Events (AE)

Reported incidences of AE with eltrombopag ranged from 48-87%. Most of the AE were considered mild. Grade 3-4 AE [21] occurred in 3-15% of patients.[13,16,18,19] Three studies reported nausea as an AE [16, 18, 19]; in two of those it was the most frequent AE (ranging from 8 – 12%).[16,18] All four studies mentioned an increase of liver aminotransferases (3-7%) and two mentioned hyperbilirubinemia (4%) [13,16] as a possible AE. In general, this event resolved quickly after discontinuation of treatment. Two studies reported progression of cataract in 4% of patients, all of them with a history of corticosteroid use.[16,19]

For romiplostim, the AE were similar. Headache, dizziness, pain in extremities and fatigue were most frequently seen. (14,17) Gernsheimer et al. reported bone marrow reticulin formation as a possible AE.[17]

Figure 2- Forest plot of comparison of platelet responses, TPO agonists vs. placebo.

Group by Drug	Study name	Statistics for each study			
		Risk ratio	Lower limit	Upper limit	p-Value
Eltrombopag	Cheng et al. (2011)	2.864	1.892	4.334	0.000
Eltrombopag	Bussel et al. (2011)	3.632	1.704	7.744	0.001
Eltrombopag	Bussel et al. (2011)	6.333	2.120	18.924	0.001
Eltrombopag	Tomiyama et al. (2011)	10.688	0.701	162.896	0.088
Eltrombopag		3.317	2.356	4.671	0.000
Romiplostim	Gernsheimer et al. (2008)	11.639	3.895	34.780	0.000
Romiplostim	Shirasugi et al. (2011)	11.455	1.750	74.973	0.011
Romiplostim		11.592	4.502	29.849	0.000
Overall		3.834	2.779	5.290	0.000



Heterogeneity overall: Q=8.532, df=5, p=0.129, I2= 41.4%
Heterogeneity eltrombopag subgroup: Q=2.588, df=3, p=0.460, I2=0%
Heterogeneity romiplostim subgroup: Q=0.000, df=1, p=0.0989, I2=0%

Discussion

Our study suggests that TPO-RAs are effective at increasing platelet counts and decreasing the risk of bleeding episodes as compared to the standard of care. Furthermore, they are relatively safe, with mostly mild adverse events. No difference in efficacy was found between splenectomized and non-splenectomized individuals, as 4 out of 5 studies on this topic showed similar platelet responses in both groups. Currently, TPO-RAs are only registered for treatment of splenectomized patients who are unresponsive to other treatment and non-splenectomized patients in whom surgery is contraindicated.[22] The results of our review suggest that a broader use of TPO-RAs is possible.

Furthermore, our study suggests that TPO-RAs are also effective at reducing bleeding episodes in patients with chronic ITP, when compared to placebo. All six studies on this subject reported fewer significant bleeding episodes. Although only three studies provided statistical evidence, these studies are all of high methodological quality. Stasi et al. found that treatment with romiplostim decreased the rate of bleeding relative to the standard of care as well. However, this study had some methodological issues, and therefore did not provide sufficient evidence to conclude that romiplostim is also superior to the standard of care regarding fewer bleeding episodes.

TPO-RAs are generally well tolerated, with grade 3-4 AE occurring in 3-15% of patients. Liver function abnormalities were seen in 3-7% of eltrombopag-treated patients. We therefore recommend monitoring liver aminotransferases and serum bilirubin before and during treatment. One study mentioned bone marrow fibrosis as a possible AE of romiplostim. This has been reported in other studies as well, which we did not include in this review, and has raised some concern. These studies showed that romiplostim induces bone marrow hypercellularity and mild reticulin fibrosis in some patients. No collagen deposition was seen. [23,24] As these short-term studies only included small numbers of patients, larger, long-term, prospective studies are needed to establish whether bone marrow reticulin deposition will be a clinically significant and relevant limitation to the usage of romiplostim.

Platelet responses are sustained as long as the treatment is given. After discontinuation of treatment, platelet counts dropped to values near baseline in all studies. This means that long-term – even lifelong – treatment would be necessary. Long-term data are lacking, so further research, with a longer follow-up period of at least 5 years, is required to establish whether TPO-RAs are effective and safe with long-term use as well.

Limitations

The conclusions of our study cannot be generalized to the entire population, as we only included articles that studied patients 18 years and older. Our conclusions are therefore applicable only to adult patients. We did so, because ITP in children is quite different from ITP in adults. In children, ITP appears in an acute form most of the times, and even in chronic ITP, spontaneous remissions occur in one-third to half of children, independent of the administered treatment.[25,26]

We cannot make any conclusions on the efficacy and safety of TPO-RAs relative to the current standard of care. We included no studies on the rates of platelet responses and only one on bleeding episodes compared with standard of care, but as mentioned before, the latter was of poor methodological quality.

Although the study populations are comparable in general, differences do exist that might have confounded our results. Stasi et al. also included patients with platelet counts >30 x 10^9/L, although treatment is not routinely given in those patients.[3] Furthermore, different dosage schedules were used in the different studies, because adjustments could be made based on platelet responses. However, this reflects the use of TPO-RAs in practice, where 1-10 µg/kg of romiplostim is given subcutaneously once weekly with or without 50-75 mg of eltrombopag orally once daily.[27] The lower starting dosage in the study of Tomiyama et al. also corresponds with general practice because of differences in the pharmacokinetics between patients of East Asian descent and those of non-East Asian descent. The area under the curve (AUC) was almost two times larger in the East Asian group, which implies that lower starting dosages can be effective in those patients. [28] For that reason the recommended starting dose in East Asians is 25 mg once daily, according to the FDA.[29]

Furthermore, the study populations of the included articles in this review were relatively small, especially those of Tomiyama et al. and Shirasugi et al. We suggest a multi-center trial to assess the effect of TPO-RAs in larger populations to obtain more reliable results.

Overall, we conclude that TPO-RAs are an effective and safe treatment option for ITP in adults. However, we recommend that further research be conducted, with larger study populations and long-term follow up to confirm the benefits of TPO-RAs in practice.

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Antibody-mediated rejection after kidney transplantation

an overview of current treatment options

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Abstract

Acute antibody-mediated rejection (AMR) after kidney transplantation is an uncommon but serious event, usually leading to graft loss. The current treatment modalities combine plasmapheresis and intravenous immunoglobulin (IVIg). Based on our study of the relevant literature, we compared the efficacy of drug regimens either to treat or to prevent AMR. A Pubmed search was performed using kidney transplantation, graft rejection, humoral rejection and antibody-mediated rejection as search terms.

Fourteen studies were identified. The various treatment regimens used drugs designed to combat antibody formation and/or immune modulation of the effects of the antibodies. The following treatments for AMR were used: plasmapheresis, IVIg, plasmapheresis combined with IVIg, plasmapheresis combined with IVIg and rituximab, rabbit antithymocyte globulin, bortezomib, or splenctomy. Complement inhibitor eculizumab was used in one study to prevent AMR in immunologically high risk transplant candidates.

Evidence based conclusions can not be drawn from these studies due to the lack of sufficient data, small numbers of included patients, and the extensive co-medication used, which made it impossible to determine the efficacy of a specific drug. Therefore, we suggest to perform a multi-centre RCT with a transparant design comparing the current standard therapy consisting of plasmapheresis and IVIG with eculizumab in a large cohort of patients who develop AMR looking at the graft survival and function as primary endpoints. A longer follow-up period from 5 to 10 years is desirable.

Abbreviations: AMR, antibody-mediated rejection; DSA, donor-specific antibodies; IVIg, intravenous immunoglobulin; MMF, mycophenolate mofetil; rATG, rabbit antithymocyte globulin; RCT, randomized controlled trial.

Introduction

Recently, several studies have shown that acute antibody-mediated rejection (AMR) is associated with poor prognosis following kidney transplantation.[1] Acute AMR is a rare but serious event. The risk of allograft failure among highly sensitized patients is particularly high in the first three months post transplant. The 1-year graft survival has been reported to be only 15-50% despite intensive conventional immunosuppressive therapy.[2]

Acute AMR occurs because of antibody-mediated complement activation and eventual lysis of graft endothelium. As such, AMR is associated with C4d deposition in peritubular capillaries. C4d is the complement split product resulting from the cleavage of C4b. C4d can then covalently bind to the vascular endothelium of the kidney graft.[3]

To justify the diagnosis of AMR, in case of graft dysfunction, 3 major diagnostic criteria must be in accordance with the latest Banff classification [4][5]:
1) morphological evidence of acute tissue injury;
2) immunopathological evidence of antibody activity;
and 3) serological evidence of circulating DSA or antibodies against other donor endothelial antigens. The heterogeneous clinical manifestations of AMR, extending, for example from deterioration of graft function to just only unexplained proteinuria, have been always a barrier for the transplant nephrologists to investigate the outcomes in completely homogenous comparable patient groups. Of note, C4d negative antibody mediated rejection has been recently reported, but still needs more detailed description and definition in the future.[6]

Although no strict evidence-based treatment modality for AMR is available at present, current strategies rely on a combination of drug regimen.
For instance, plasmapheresis, intravenous immunoglobulin (IVIg) and immunoadsorption, targeting removal of antibodies. Another treatment relies on plasmapheresis in combination with monoclonal anti-CD20 antibodies (rituximab) or bortezomib, targeting B cells and plasma cells. Furthermore, polyclonal antilymphocyte antibodies including rabbit antithymocyte globulin (rATG), in addition to maintenance immunosuppressive medication (tacrolimus, cyclosporine A, mycophenolate mofetil, and prednison), have been used to treat AMR (Table 1). The aim of this review was to compare the efficacy of the reported drug regimen for treatment or prevention of acute AMR in renal transplant recipients.

Method

We systematically searched Pubmed, using the search terms; kidney transplantation, graft rejection, and humoral rejection/ antibody-mediated rejection. In addition, we included the references from the meeting report of the FDA Antibody-mediated rejection workshop in 2011.[6]
With these terms the following search stream was established: ("Kidney Transplantation"[Mesh]) AND ("Graft Rejection/ therapy"[Mesh]) AND ("Immunity, Humoral"[Mesh] OR "antibody-mediated rejection"[All]).

The following inclusion criteria were applied to select the studies: published in English, based on clinical trials, and a study population of more than 5 patients. Studies were excluded if the full text was not available for Erasmus MC or LUMC students, no review or if the study concerned ABO incompatibility rejection. We also screened the references of the included studies for useful articles.

Results

Graft survival was the primary endpoint in all studies (Table 1). Only two studies [1,7] provided additional information on serum creatinine levels as the primary endpoint reflecting the actual graft function. Studies investigating prevention of AMR in a high risk population used the incidence of AMR as the primary endpoint. The majority of the population samples in the included studies consisted of Caucasians. Some studies also included minorities; i.e. black and Hispanic patients.

Studies on Treatment of AMR

Plasmapheresis/ IVIG/immunoadsorption targeting removal of antibodies or immunomodulation of the effects of antibodies
Jordan et al. treated seven patients suffering from AMR with IVIg (2 g/kg) combined with maintenance immunosuppression consisting of cyclosporine, prednisone, and azathioprine. They reported on a 4 year follow-up in this case series. Graft survival of 100% was achieved. They concluded that treating acute AMR with IVIg and maintenance triple immunosuppression was effective in treating AMR.

Table 1 - Published reports on treatment or prevention of acute AMR in kidney transplant recipients.

Publication	Treatment regimen	N	FUP	Graft survival (%)	Patient survival (%)	Serum creatinine (mg/dL)
Jordan et al. (1998)[16]	1x IVIg, CsA, Pred, and AZA	7	4 y	100	–	1.2 – 1.7
Pascual et al. (1998)[2]	4-7x PPh followed by 1x IVIg, TAC, MMF, and Pred	5	19.6 ± 5.6 m	100	100	1.2 ± 0.3
Crespo et al. (2001)[17]	5 xPPh with TAC and MMF and IVIg	19[b]	29 m	80	100	1.5 ± 0.4
Rocha et al. (2003)[9]	4 xPPh, 1-3x IVIg, Mpred pulses, CNI, MMF, and Pred[c]	16	457 ± 76 d	81	–	1.6
Shah et al. (2004)[18]	6.8x PPh every other day, 6x rATG, TAC, MMF, and Pred	7	1 y	85.7	–	1.46 ± 0.33
Becker et al. (2004)[11]	1x RTX, Mpred, rATG with or without PPh, CsA or TAC, MMF, and Pred	27[d]	605 ± 335.3 d	85[e]	77.8	0.95 ± 0.29
Böhmig et al. (2007)[10]	A: 9-14x IAf, TAC, and ALA or Pred	A: 5	2 y	A: 80	–	A: 2.2
	B: TAC and ALA or Pred[g]	B: 5		B: 20		B: 1.6
Faguer et al. (2007)[12]	3x Mpred, 4x RTX, 9x PPh, and TAC, MMF, and Pred	8	10 m	75	100	153 µmol/L
Everly et al. (2008)[7]	PPh, 4x BOR, TAC, MMF, and Pred[h]	6[i]	140 d	66.7	–	–
Lefaucheur et al. (2009)[1]	A: 4x IVIg, 3x Mpred pulses	A: 12	36 m	A: 50	–	–
	B: 4x PPh and IVIg, and 2x RTX	B: 12		B: 91.7		
	Maintenance IS in both groups: TAC or CsA, MMF, and Pred					
Brown et al. (2009)[8]	8.1x PPh, TAC or EVE, MMF, and Pred	18	5 y	78	93	130 µmol/L
Stegall et al. (2011)[14]	A: ECU, induction therapy with rATG, TAC, MMF, and Pred	A: 26	A: 11.9 m	A: 100	–	–
	B: historical group: 4-14x PPh, induction therapy with rATG, TAC, MMF, and Pred	B: 51	B: 48.8 m	B: 96		
Liefeldt et al. (2012)[15]	A: CsA, twice daily MS, and Pred	A: 66	1273 d	–	A: 95.4	–
	B: EVE, twice daily MS, and Pred	B: 61			B: 98.4	
Tzvetanov et al. (2012)[13]	7.2x PPh followed by IVIg, splenectomy as rescue therapy, Maintenance IS : TAC, MMF, and Pred	11	25.8 ± 19 m	81.8	90.9	2.8 ± 1.5

FUP = Follow-up; ACR = acute cellular rejection; AMR = antibody-mediated rejection; DSA = donor-specific antibodies; GFR = glomerular filtration rate; IV = intravenous; IVIg = intravenous immunoglobulin; MMF = mycophenolate mofetil; PPh = plasmapheresis; PTC = peritubular capillaries; rATG = rabbit antithymocyte globulin; CsA = cyclosporine; Pred = prednisone; AZA = azathioprine; TAC = tacrolimus; CNI = calcineurin inhibitor; RTX= rituximab; IA = immunoadsorption; Mpred= methylprednisolone; ALA = anti-lymphocyte antibody; BOR = bortezomib, EVE = everolimus; ECU = eculizumab; MS = mycophenolate sodium
y=years; m=months; d=days; Maintenance IS: Immunosuppression (TAC, MMF and Pred)

[f] First 3 plasmapheresis once daily on consecutive days followed by sessions with intervals of 3 days for a period up to 6 weeks
[g] Patients in group B had the option of immunoadsorption rescue after 3 weeks
[h] Some patients also received rATG, rituximab, IVIg, or a combination of those treatments
[i] Patients with mixed rejection; AMR & ACR

In the observational study of Brown et al., 18 patients were included. Plasmapheresis (up to 8 times), in combination with maintenance immunosuppression consisting of tacrolimus or everolimus, mycophenolate mofetil (MMF) and prednisone, was the therapy of choice. After a follow-up period of 5 years, the graft survival appeared to be 78%. The authors concluded that plasmapheresis was successful, with an excellent long-term graft and patient survival (93%).[8]

In another study performed in 1998, plasmapheresis (4-7 times) in combination with tacrolimus (0.14 g/kg/day) and MMF (2 g/day) was used to treat AMR. The authors concluded that this treatment is effective in preventing kidney graft loss due to AMR. The patients were followed for 19.6 ± 5.6 months. No graft was lost. They postulated that this mode of therapy is effective for patients who develop circulating DSA after transplantation (n= 1/5).[2]

A similar clinical trial published in 2001 reported on kidney transplant recipients who received five cycles of plasmapheresis together with tacrolimus (0.11 mg/kg/day) and IVIg (0.4 /day). This study showed that acute AMR was reversible in 90% of patients. The authors concluded that the low dose of IVIg might have had an effect on the reversibility of AMR and on graft survival.[12] Rocha et al., on the other hand, used high dose IVIg (2 g/kg) in combination with plasmapheresis (4 cycles) to treat acute AMR. In their study the average one-year graft survival was 81%. AMR was strongly related to a higher occurrence of delayed graft function immediately after transplantation.[9]

In 2007, a RCT was performed to investigate the efficacy of immunoadsorption in treatment of acute AMR. In this study 10 patients were included and randomized as 1/1. The treatment arm (group A) underwent 9-14 immunoadsorption treatments next to standard treatment, whereas the control group (group B) was only treated with tacrolimus, prednisone and anti-lymphocyte antibodies. After inclusion of 5 patients in each arm, the investigators had to terminate the study because of the evidently superior clinical benefit of immunoadsorption therapy. Graft survival in group A was 80%, compared to 20 % in the control group. In immuno-adsorption group all episodes of AMR were completely reversible compared to only 20% of rejection episodes in group B.The patients were then followed for two years. The authors concluded that immunoadsorption is capable of improving graft function within three weeks after starting the treatment and is superior to the treatment in control group in every aspect.[10]

Lefaucheur et al. investigated two treatment options; high dose IVIg (2g/kg) with plasmapheresis (4 times), The 24 patients were followed for 36 months. Patients treated with plasmapheresis showed a significantly better graft survival (p= 0.02). It was concluded that plasmapheresis is superior to high dose IVIg alone in treating acute AMR.[1]

Rituximab and bortezomib targeting B cells and plasma cells

Two studies evaluated the effect of rituximab on acute AMR. In the study of Becker et al., 27 patients were included. The patients received rituximab (375 mg/m2), intravenous methylprednisolon (500 mg/day), rATG (1.5 mg/kg/day) in combination with plasmapheresis. The follow-up time was 605 ± 335.3 days and the graft survival 85%.

In the study of Faguer et al, 8 patients were included. The patients received rituximab (375 mg/m2) in combination with methylprednisolone (10mg/kg/day) and plasmaptheresis. Graft survival rate was 75%.

Altogether, both studies showed a similar graft survival. The first study concluded that rituximab might be effective in treating acute AMR, which is unresponsive to steroids [11], while the second study concluded that rituximab was effective in reversal of acute AMR.[12]

Everly et al., studied the effects of bortezomib on treating mixed rejection types after kidney transplantation. This study 6 included patients, which received bortezomib (1.5 mg/m2) together with plasmapheresis as treatment regimen. The patients were followed for 140 days and the graft survival rate was 66.7%.The results led to the their conclusion was that bortezomib is effective in treating mixed rejection types with and that it has minimal toxicity.[7]

Other approaches; ATG, splenectomy and eculizumab

Another study on the effects of rATG in treatment of acute AMR showed no difference in survival between patients with or without AMR, indicating a good long-term survival.[15] Seven patients with AMR were included and received 6.8 times plasmapheresis and 6 times rATG (0.75mg/kg/day). The patients were followed for one year. Graft survival was 85.7%.

A clinical trial investigated the use of splenectomy as a rescue therapy for acute AMR. The 11 patients included in the study, who did not respond to the standard therapy consisting of plasmapheresis and IVIg (100mg/kg), received the rescue therapy. Patients were followed for 25.8 ± 19 months and the graft survival appeared to be 81.8%. The authors concluded that splenectomy can be used to treat patients who are unresponsive to the applied therapies. Unfortunately, this study was still not published in a pear reviewed journal.[13]

Studies on prevention of AMR

A recent case-control study concerned prevention of acute AMR using a historical control group. The authors examined the efficacy of complement inhibition with eculizumab, for the prevention AMR in immunologically high-risk recipients transplanted with a donor kidney after desensitization therapy. The incidence of biopsy-proven AMR in 26 highly sensitized recipients, who received eculizumab according to a special scheme starting at a pre-transplant time point, was compared to a historical control group of 51 sensitized patients treated with a similar plasma exchange-based protocol without eculizumab. The incidence of AMR was 7.7% (2/26) in the eculizumab group compared to 41.2% (21/51) in the control group (p = 0.0031). Stegall et al. concluded that eculizumab seems to be an effective therapy, because it prevented the development of AMR in highly sensitized patients compared to the controls; this result was statistically significant.[14]

Liefeldt et al. performed a post hoc analysis on the relationship between the use of everolimus vs. cyclosporine and the occurrence of donor-specific antibodies. In this study, 127 patients were included and the follow-up was 1273 days.

Group A received cyclosporine (100-150 ng/ml) and group B received everolimus (6-10 ng/ml). Patients treated with everolimus developed more acute AMR and new DSA than those treated with cyclosporine (p=0.048). The authors concluded that everolimus is associated with an increased risk for AMR development and suggested limiting the use of everolimus to patients with low immunological risk.[15]

Discussion

The treatment regimen published by Böhmig et al., a high quality RCT, seems to be a promising treatment for AMR. In this study the effect of immunoadsorption in combination with a standard immunosuppressive therapy was compared to the standard therapy alone. The function of immunoadsorption is to remove the DSA in the blood of the recipient. The results of this study showed that the reversibility of the rejection was significantly different between the two study groups. The study had to be terminated after inclusion of 5 patients in each arm because of superiority of treatment arm with i immunoadsorption. All the patients treated with immunoadsorption showed reversal of the rejection and the graft survival was also better. Based on results of this well designed study, the proof of concept is delivered that removal of antibodies might be an essential step in the treatment of AMR.

The treatment regimen published by Stegall et al., seems to be an effective therapy to prevent the occurrence of AMR in high-risk patients. They used eculizumab in combination with induction and maintenance immunosuppressive therapy. The results showed a significant difference in the incidence of acute AMR, with less AMR occurring in the patients treated with eculizumab as compared to almost 40% AMR incidence in their historical control group. Therefore, eculizumab might be effective in preventing the occurrence of AMR.[14]

The treatment regimen published by Everly et al., could also be considered as a possible treatment option for AMR. Bortezomib has an effect on the plasma cells, the source cells for the production of the DSA. However, the side effects and high level of toxicity of bortezomib is a restriction. The agents inhibiting plasma cell activity with less severe side effects should preferably be investigated as soon as they become available.[7]

Limitations

Based on findings reported in the literature, evidence-based conclusions cannot be drawn from the aforementioned studies on treatment of AMR because of the lack of high-quality data, the small numbers of included patients and extensive co-medication used, which makes it very hard to determine the efficacy of a specific drug. Most of the studies were clinical series without a control group. Some included a historical control group. Lack of RCT design makes it difficult to assess and compare the efficacy of different treatment modalities. The results should therefore be interpreted with caution. Most of these clinical trials included only a small number of patients, i.e. the average number included was 7. In general the end points were well defined and relevant: kidney graft survival and function. Furthermore, the extensive and variable co-medication schemes limit the interpretation of the data obtained, which makes it impossible to draw any solid conclusions.

Concluding remarks

As it is clear, no evidence-based conclusions can be drawn about the superior therapy for treatment or prevention of AMR. More research is required to determine which treatment option is the most effective modality in treating AMR. However, it is our belief that eculizumab is effective in preventing the occurrence of AMR. Therefore, we suggest that a multi-centre RCT be performed with a transparant design comparing the current standard therapy consisting of plasmapheresis and IVIg, with eculizumab in a large cohort of patients who develop AMR. Graft survival and function should be investigated as primary endpoints with a longer follow-up period of 5-10 years. All patients should receive maintenance immunosuppressive medication in the same manner.

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Placebo, chance for optimal treatment or threat What is the doctor-patient relationship worth?

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Introduction

The term placebo has been used since the 1950s. A placebo can be seen as an inert substance and the placebo effect follows after the dose.[1] When prescribing a placebo, the practitioner records the agent as a drug, but it isn't. Patients think they are taking a drug to treat their illness/ pain, assuming that the drug will have a beneficial effect. We can see this process as the positive energy we draw from ourselves. Evidence is increasing that the placebo effect is a genuine psychobiological event, attributable to the therapeutic context in which the patient is being treated at that moment.[1] However, this raises an important issue: can doctors ethically prescribe placebos to patients? Patients assume that the drug will make them better or will alleviate their symptoms, but in fact the efficacy of placebos has not been effectively proven in practice. Some Randomized Controlled Trials (RCTs) have indicated that placebos are effective. However in most of these RCTs a treatment group is compared to a group receiving placebo. This means that both groups know that there is a chance they will receive a placebo. This is much different from a doctor prescribing a placebo in practice and patients not being aware of it. If patients discover that they have been tricked, they may feel cheated and may no longer have any confidence in the practitioner.

As more and more people become aware of the prescription of placebos, this could cause major changes within our health care system. The population could lose confidence in doctors and other caregivers. The doctor-patient relationship could be in danger. At this time I think it's unethical that the autonomy of the patients is taken from them this way. It can be seen as a form of paternalism where decisions that affect patients are taken – without their knowledge – by someone else. In this essay I focus mainly on the dilemma of prescribing placebos to patients who have unexplained somatic symptoms. Is it ethical to give placebos to such patients? Is there a difference if patients are informed that they have a chance of getting a placebo? And what is the influence of a positive or negative context in which the "placebo" is prescribed?

Medical scientific progress

In 2006 "The American Medical Association's Council on Ethical and Judicial Affairs" took a position against the use of placebos in clinical practice.[2] To ethically defend the use of placebos, their effectiveness must be proven first.[3]

It has been shown that only a small number of doctors in the USA prescribe inert pills and injections (that comply with the formal definition of a placebo). However, 50% of the doctors prescribe medications which they expect will have no effect on the condition of the patient and thus essentially serve as a placebo.[4] If we look at the improvement in the subjective symptoms it is therefore important to find a way to make use of this placebo effect and avoid patient- deception.
Previous research has shown that the placebo effect is clinically significant in Irritable Bowel Syndrome (IBS).[1] No diagnostic tests and no effective treatments are available for IBS.[3] One study showed that the use of an "open-label"- placebo, which were defined as openly described inert interventions delivered with a plausible rational, is an effective treatment in IBS. In comparison to patients who received no treatment, patients with an open-label placebo scored much better with respect to symptom improvement. This study ran from 2009 until April 2010. However, it is debatable whether open-label placebo is a real form of placebo treatment.[4]

In a recent meta-analysis, all 73 RCTs that were eligible for the study (a total of 8364 patients with IBS) found a placebo response rate of 37.5%.[5] In a Randomized Controlled Trial, researchers compare treatment groups with control groups not receiving treatment (as in a placebo-controlled study).
The placebo effect reported in RCTs is controversial, because the positive effect in the placebo group is not necessarily a psychosocial effect. It may be that this is the natural course of the disease, the extent to which the symptoms fluctuate, regression to the mean or response bias because the patient shows subjective symptoms. Sometimes it's impossible to exclude that the effect is not caused by other concomitant therapies.[1] This has to do with the assumption of ceteris paribus, it's not always possible to guarantee that the other parameters remain the same.

Another question we must ask is whether a placebo has side effects, called the nocebo phenomenon. Here we should distinguish between events occurring in getting the drug (regardless of cause), and side effects that can be directly attributed to the drug (the cause is the drug itself).[6] It is a distinction between the expected negative effect and side effects of placebo. The nocebo effect is getting diseases or symptoms, in combination with negative expectations and associated with a particular emotional state.[7]

From a psychological standpoint many mechanisms contribute to the placebo effect. Patients who receive a placebo have certain expectations of the response in the future. It has been shown that if a positive expectation is aroused in a patient, the patient responds better to the placebo.[1][8][9] [10] Another mechanism is due to classical conditioning [11]. Repeated associations between a neutral stimulus and an active drug may result in the ability to induce an effect through a neutral stimulus, similar to that of the active drug. In addition, previous experiences and social observations also affect the potential placebo effect.[12]

Another study looked at patient and therapist influences on the operation of the placebo effect in IBS. The study focused on the characteristics of patients and therapists and their personal interaction. The conclusion was that gender (female) and personality of the patients (outgoing, pleasant and open to new experiences) had an influence on the placebo effect, but only in the group where there was a warm and empathic interaction between patients and therapists.[13] Besides IBS a possible beneficial placebo effect has been reported for a number of symptoms/ conditions.(table 1).

Table 1 - Complaints whereby placebo could be useful.

A. Hróbjartsson and P.C. Gotzsche (14)	W. Hauser et al. (15)
pain	fibromyalgia syndrome (FMS)
nausea	peripheral diabetic neuropathy (DPN)
asthma	
phobia	

Ethical aspects

Patients think they feel better when they take a pill (which is prescribed by someone with extensive knowledge and experience), but in fact, its efficacy is not known. Regardless of whether patients feel better from taking a placebo, the ethical dilemma to deal with is deception. Doctors want the best for their patients, with a positive outcome, but this does not justify ‘’cheating”. In principle, lying and not telling the whole truth can be seen as two separate things. For example, doctors don’t tell patients all the possible symptoms of a specific disease, but provide only the most important ones and omit the others. However, this omission is probably unconscious; it is information that is in the background and not directly of the utmost importance. Doctors have good intentions and will come back to this aspect later on.
They can justify why they did not tell everything immediately. Therefore, this is totally different than prescribing a placebo. The principles of beneficence and doing no harm also play a part. If a doctor prescribes a placebo to a patient, he/she intends to do the best for the patient. Yet no one has the right to determine that do well, and not harm are beyond the responsibility not to keep the patient in state of deception; leaving the patient in state of deception is worse than not offering the opportunity of placebo treatment. In addition, at the time that the patient finds out that he/she is getting a placebo, there is no longer any good: it’s more harm. This is because the patient has lost confidence in the practitioner, which is often to the detriment of the patient because the treatment relationship is damaged.[16]

Another problem is that patients can’t give informed consent if they are not aware of what they get. They didn’t receive the information that allows them to make a rational decision. In this way patients have no influence in their treatment.[16] Besides, by giving a placebo, practitioners violate the right to autonomy. In fact patients are not aware of their options. Autonomy means that patients are informed of their condition, that their questions are answered and that the treatment options, including risks and benefits, are discussed.[17] However, the patients may also prefer to remain in state of deception in this way (with a placebo) instead of knowing the truth. Of course we can’t estimate this beforehand. It’s clear that through deception they couldn’t make their own choice.

Conclusions

On the first look, placebo prescription seems to be a good treatment for certain groups of people. It's possible that placebo has a positive effect, that there's a reduction of symptoms and the patients feel better. If we're ever going to see placebo as a real treatment, we must take some factors into account. The most important is to inform the patient very well.

The first option is not to tell patients that they have a somatically unexplained physical symptom; instead tell them a treatment is available. Doctors prescribe a drug, but they tell them that the effect of the drug is not known. What do you put at the box (containing the so-called drug) that the patient will get at the pharmacy? They can invent a name for the drug, but an internet search will reveal that this drug does not exist. In addition, the name of this placebo will at least be known by healthcare workers. In this way, if it's impossible to prevent patients from finding out that they have received a placebo, to what extent is the effect of placebo still guaranteed?

The second option is to proceed as outlined above, but then explain that they largely concern a psychosocial effect and related physical changes and possible improvement of somatic complaints. It may be that patients in this way do not make a connection with a placebo. However, the effects of a placebo (positive and negative) are not always predictable or known. We are unable, as with many other medicines, display the exact effect and side effects.

A third option is to tell patients who have unexplained physical symptoms that they can choose a placebo treatment. The placebo is discussed in a positive context. It is mentioned that a positive effect is not guaranteed, but that many positive results are known ("open-label placebo"). This possible effect, however, depends on several factors, but it should be emphasized that it can have a positive impact on individual patients. However, the question remains of whether an open-label placebo can be seen as having a placebo effect.

In my opinion we are not ready to prescribe placebos in clinical practice. There are still too many drawbacks for prescribing placebos and the evidence is not unequivocal. A study has demonstrated that patients with IBS with an "open-label" placebo had better outcomes than no treatment, but also many people with "open label'' placebos experienced no effect. I think the second of the above options is best. Doctors do not lie to patients, but in fact withhold a little information. I believe that option three is impossible to apply. Supposing that we would apply the second option in the Netherlands, then we should have a clear policy with respect to this form of placebo. I think we should offer this option to all patients with an unexplained somatic disorder. Then we can give patients the opportunity to choose freely. For me, confidence in the healthcare system is essential. The doctor-patient relationship is crucial.

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Solarium use? Only 18 years and older!

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Introduction

In the Netherlands, a political discussion is ongoing about whether or not to forbid the use of solariums for people under the age of 18. This discussion has become urgent due to the increasing popularity of tanning devices and knowledge about the potential risks of ultraviolet radiation.[1] Ultraviolet (UV) radiation is a known cause of skin cancer and is emitted by the sun and artificial tanning devices.[2] For this reason, the Dutch government should prohibit the use of solariums for people under the age of 18. In this opinion paper the motives, pros and cons of solarium use and other reasons why solarium use should be prohibited are covered.

State of affairs

Banning the use of solariums for people under 18 years of age is supported by the World Health Organisation (WHO), the American Society of Paediatrics (AAP), the American Society of Dermatology (AAD), the American Medical Association (AMA) and other organizations.[3] These organizations believe that this ban can save lives and is essential for preventing skin cancer.[3]

What are the motives nowadays for to use a solarium? A recent study revealed the following reasons: to look better, to feel more confident, to look healthier and to emulate tanned celebrities.[4] This applies particularly to young people in Europe; in much of Asia, for example, people traditionally prefer a white skin, so they are less likely to use a solarium. They even sell a lot of skin whitening products in parts of Asia. Also, people with dark skin probably would not use a solarium to get a tan.

Many youngsters are unaware of the health risks of UV radiation.[4] It is a misconception that a tanned skin protects you from sunburn.[4] A tan is a sign of DNA damage and an attempt of the body to repair DNA damage in the skin.[4] Burning of the skin is also a sign of DNA damage. During tanning sessions, 57% of the teenagers burn at least once.[5]

For men in their twenties, melanoma is the third most common form of cancer, for women in their twenties the second most common form.[3] The increasing popularity of solarium use is associated with increased incidence of melanoma.[3]

No solarium use under 18

Indoor tanning and skin cancer

The incidence of basal cell carcinoma, squamous cell carcinoma and the potentially fatal melanoma is increasing, particularly among young women.[3,6,7] A risk factor for malignant melanoma is artificial tanning, and probably the same is true for basal cell carcinoma and squamous cell carcinoma.[8,9] The use of a solarium before 35 years of age is associated with an increased risk of developing malignant melanoma of 59%.[10] People who start tanning at a young age, have a greater risk of skin cancer.[2,11]

Melanoma

Most mortality from skin cancer is caused by melanoma, and the mortality rate for melanoma is high.[4,12] The risk of melanoma increases in proportion to the number of tanning sessions.[10] In one study, the researchers compared a group of people who had used a solarium to a group of people who had never done this. The group that had used a solarium was 41% more likely to develop melanoma. More than ten tanning sessions doubled the risk of melanoma, compared to no session.[11]

The risk of melanoma is also greater when the first solarium session was at a younger age.[10,11] People who have used a solarium during high school or continuing education, or between the ages of 25 and 35, also have an increased risk of basal cell carcinoma.[13] The sensitive period for long-term effects of UV radiation, such as skin cancer, seems to be the period up to 18 years.[14]

Benefits

Solarium use also has benefits. The use of a solarium increases the blood levels of vitamin D.[15] This UV exposure might even result in fewer internal cancers. Other benefits of UV exposure include protection against infectious diseases, diabetes, multiple sclerosis and mental disorders.[15] Still, the risk of skin cancer increases with solarium use.[15]

Prevention

Providing information and thus awareness about the risk of the use of solariums can possibly prevent children from using a solarium or prevent their parents from allowing its use.[16]

However, a large group remains unaffected by the information on pros and cons of solarium use. Therefore, the government should ban children from using solariums.

Pediatricians also play an important role in the prevention of solarium use. Their task is to inform their patients and the parents of the patients about the risks.[6] This could help to prevent morbidity and mortality from skin cancer.[6] A ban on the use of solariums for people under 18 years old would reinforce this goal.

Ethical principle

But why should the ban be at 18 years and not for example, 16 of 35 years of age? Based on the ethical principle of the right to autonomy, people have the right to self-determination. The most sensitive period for long-term effects of UV radiation, such as skin cancer, is the period up to 18 years.[14] Therefore it is justified to adopt a law which states that people under the age of 18 are not allowed to use a solarium, despite the right to autonomy that they have according to the law on medical treatment agreement (Dutch: WGBO: Wet op de geneeskundige behandelingsovereenkomst).[17] Such laws also apply to other potentially hazardous activities, such as banning sales of alcohol to people under 18 years. The same logic should apply to the use of solariums. Both are harmful and can give health problems in the future.[18]

Furthermore, an age limit of 35 years could be suggested. Up to this age there clearly is an increased risk of melanoma when a solarium is used.[10,19] However, this would imply that people can only decide about their own bodies after 35 years of age, which would violate the right to autonomy. We may also assume that people of 18 years and older can decide for themselves and are well aware of the health risks. Apparently, people under 18 years are not aware of the health risks, and are therefore not capable of making a decision about whether or not to use a solarium.[4]

Conclusion

Taking into account the above, there should be a legal prohibition in the Netherlands on the use of solariums applying to people under the age of 18. Solarium use is very popular, especially among young women. Meanwhile the incidence of skin cancer is increasing.

The age limit should be set at 18 years because the most sensitive period to develop skin cancer later in life is the period up to 18 years. Pediatricians play an important role in preventing morbidity and mortality from skin cancer. Their task is to inform patients and their parents. A legal ban could prevent many cases of skin cancer.

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Case Report

Brugada ECG pattern associated with fever and hypokalemia

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Summary

The Brugada syndrome is a channelopathy characterized by typical ST-segment elevations in the precordial leads, also known as the Brugada pattern. This pattern is associated with an increased risk of sudden cardiac death due to ventricular fibrillation. In this case report we describe a 72-year old man who developed a Brugada ECG pattern during an increase of body temperature and hypokalemia. After reduction of body temperature and in the presence of persistent moderate hypokalemia, the Brugada ECG pattern only partly resolved.

A 72-year old Caucasian male was hospitalized for treatment of acute myeloid leukemia and non-Hodgkin lymphoma with chemotherapy. He developed fever episodes of 38-39 °C of unknown origin. He had no history of cardiovascular diseases, except hypertension. The surface electrocardiograms (ECG) of the past two months revealed sinus tachycardia, most likely due to anemia caused by the malignancies, and incomplete right bundle branch block (Figure 1A). His ECG altered when a body temperature of 40.3 °C was reached; ST-segment abnormalities developed in precordial leads V1 and V2, consistent with a Brugada type 1 morphology (Figure 1B). He did not use any medication known for triggering Brugada pattern in susceptible patients. During this fever episode, severe diarrhea caused hypokalemia (3.1 mmol/l). Previous serum potassium concentrations of 3.3 mmol/l were not associated with ECG abnormalities. Antibiotics and potassium supplements were given and the ST-segment elevations (only) partly resolved (Figure 1C).

Figure 1 ECG before, during and after the fever peak

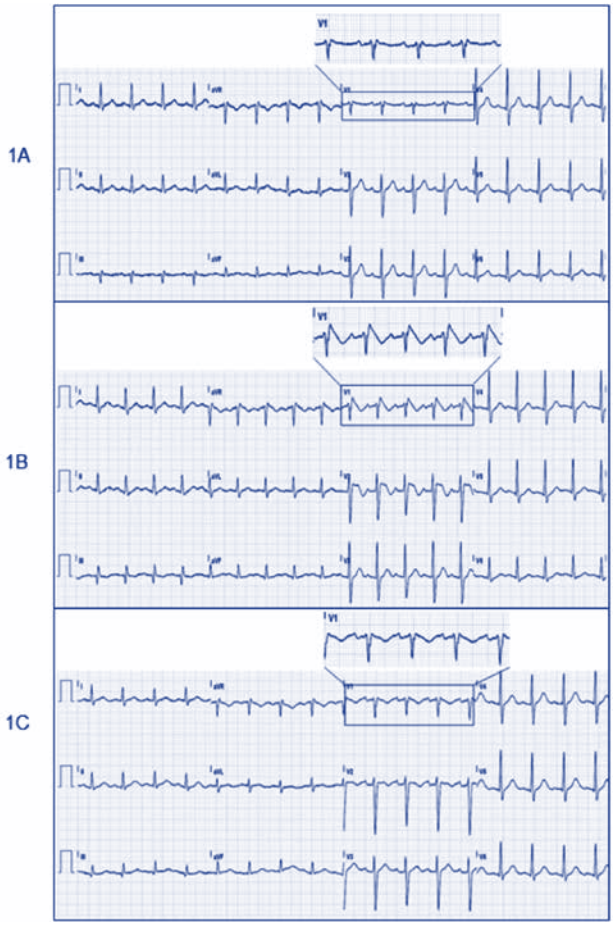

Figure legend

Fig. 1A ECG during hospitalization revealed sinus tachycardia and incomplete right bundle branch block; patient body temperature was 37.3 °C, potassium 3.8 mmol/l.

Fig. 1B ECG recorded 2 days later. Body temperature reached up to 40.3 °C and there was a moderate hypokalemia. Precordial leads V1 and V2 revealed a coved
ST-segment, consistent with a Brugada pattern type 1.

Fig. 1C ECG recorded 6 days after the start of potassium supplementation. Fever episodes (< 39.5 °C) and hypokalemia (2,9 mmol/l) were still present. The ECG abnormalities however, only partly resolved.

A moderate hypokalemia between 2.5-3.0 mmol/l persisted for two weeks despite supplementation. Fever episodes also persisted, although the body temperature remained below 39.5 °C. The slight ST-segment abnormalities remained unaltered. After 8 weeks, the patient died of his hemato-oncological diseases.

Discussion

We described a 72-year old Caucasian male known with acute myeloid leukemia and non-Hodgkin lymphoma who presented with fever episodes, hypokalemia and a Brugada ECG pattern. The Brugada syndrome (BS) is a channelopathy of the sodium channels characterized by typical ST-segment elevations in the precordial leads; it is estimated to be responsible for at least 4% of all sudden deaths.[1] So far, only the sodium channel SCN5A gene is associated with BS, while this is found in only 18-30 of the Brugada patients.[1] It is most likely there are still other yet unknown mutations in the cardiac sodium channels responsible for BS, explaining why also some SCN5A-negative patients have Brugada-like ECG alterations. It is unknown if there is a risk difference between BS with or without SCN5A mutation. Juntilla et al. demonstrated that a Brugada ECG pattern caused by fever, electrolyte imbalances or drug overdose should be considered a risk factor for developing ventricular tachyarrhythmia for both SCN5A-positive and –negative patients.[2] It is therefore essential to identify patients at risk and treat such provoking factors as soon as possible.

Hypokalemia is associated with Brugada ECG patterns and ventricular arrhythmia's [3,4] but the pathogenesis is not clear yet. Araki et al. described a patient presenting with hypokalemia (2.9 mmol/l), coved ST-segment elevation (Brugada type 1) and ventricular tachyarrhythmia's. After potassium supplementation the ST-segment changed to saddle-back configuration (Brugada type 2) and the ventricular tachyarrhythmia's disappeared.[3] In a canine model, it was demonstrated that loss of the action potential dome due to transient outward current (Ito)–mediated phase 1 of potassium in the right ventricular epicardium but not endocardium gave rise to a transmural voltage gradient that underlies ST-segment elevation, similar to that in BS patients.[5] Hence, hypokalemia may increase transmural dispersion of repolarization in the right ventricle giving rise to a Brugada ECG pattern.[4]

Fever is also known for inducing Brugada-type ECG patterns and ventricular tachyarrhythmia's.[2,6] In mammalian cells, a mutation in the sodium channel SCN5A resulted in an increased temperature sensitivity, giving rise to a faster decay of the sodium current during depolarization at higher temperatures.[7]

However, the exact relation between fever or hypokalemia, Brugada ECG pattern alterations and ventricular tachyarrhythmia's is not yet clear and additional (unknown) factors may play a role, explaining why fever and/or hypokalemia may be important in one patient, but not in the other. For example, Saura et al. described a febrile patient (body temperature up to 38.8 °C) without medical antecedents with Brugada ECG pattern type 1 which disappeared simultaneously with the fever.[8] Another article described that restoration of serum potassium in a patient, presenting with cardiac arrest, fever (39.2 °C), hypokalemia (3.0 mEq/l) and coved ST-segment elevations, resulted in ST-segment normalization irrespective of the persisting febrile state.[9]

These cases are contradictory: the febrile state is the cause of the Brugada pattern in the first case, but seems to have no influence in the second case.

Based on findings in our patient and data from literature we hypothesize that the patient developed a Brugada ECG pattern due to a combination of both elevated body temperature up to 40.3 °C and hypokalemia (2.5-3.1 mmol/l). Fever episodes alone were not enough to induce a Brugada ECG pattern, just as only moderate hypokalemia. We were unable to differentiate the effects of fever and hypokalemia as they were simultaneously present.

Apparently, lowering of the body temperature and potassium supplementation were effective as the Brugada pattern partly resolved. However, both fever and hypokalemia persisted, although to a lesser extent, most likely explaining why the Brugada ECG pattern only partly resolved. We hypothesize that the ECG alterations would have disappeared with normalization of body temperature, potassium levels or both. Because of his clinical condition, we did not further examine this patient to establish whether he had BS.

This case describes one of the various circumstances in which a Brugada pattern can occur. To our knowledge, a patient with Brugada pattern only with simultaneous fever and hypokalemia has not been described before. We consider it cost-ineffective to screen all patients with fever and/or hypokalemia for BS, due to low incidence. However, clinicians should be alert when arrhythmia's occur.

In conclusion, we described a 72-year old male with a Brugada ECG pattern due to a combination of both elevated body temperature and hypokalemia.

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Extended abstract

Evaluating the value of pre- and intra-operative adjuncts for the resection of hemispheric low grade gliomas

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Background

Low grade gliomas (LGGs) mainly affect young people (median age: 35 years), and induce seizures as the most common clinical presentation. Incidence of LGGs varies between 0.10 and 0.46 per 100,000 population in the United States, with a cumulative incidence of ~ 0.9 per 100,000 people. Diffuse hemispheric low grade gliomas include World Health Organization (WHO) grade II astrocytomas, oligodendrogliomas, and oligoastrocytomas. These gliomas account for 30% of all gliomas and are characterized by a continuous growth and progression to anaplastic transformation. The cumulative 10 year survival rate among patients newly diagnosed with supratentorial LGGs is 42.6%.

Total resection of LGGs remains a challenge, because LGG borders are difficult to discriminate from surrounding healthy brain tissue during surgery. LGGs infiltrated to the so-called eloquent brain regions, meaning involvement of basal ganglia, white matter tracts, sensorimotor regions and language cortices (like Wernicke's and Broca's area) are irresectable, because of high risk of causing post-operative neurological deficits.

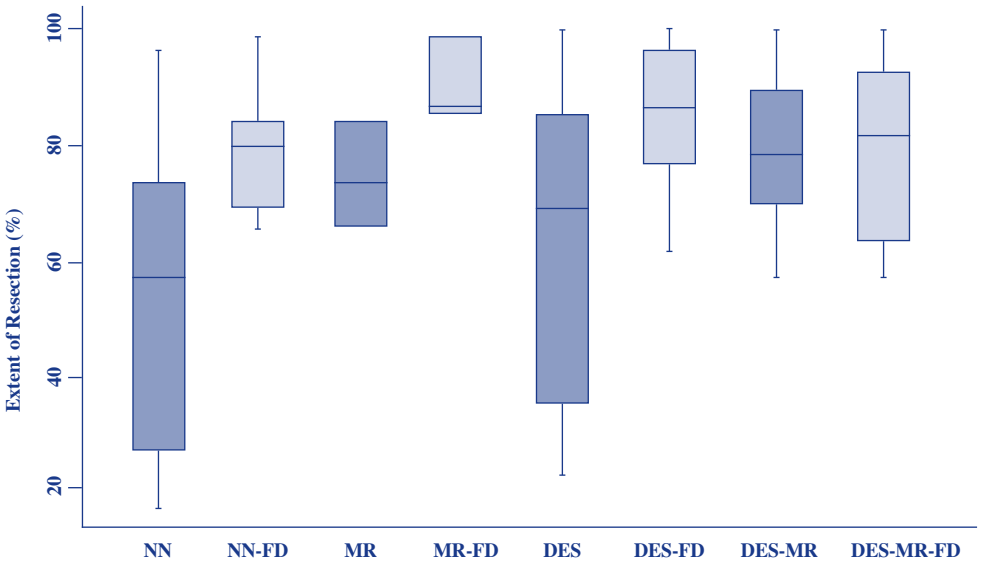
To achieve maximal resection with minimal risk of postoperative neurological morbidity, different neurosurgical adjuncts are being used during low grade glioma (LGG) surgery. The goal of this study was to investigate the effect of pre- and intra-operative adjuncts on the extent of resection (EOR) of hemispheric LGGS located both in eloquent and non-eloquent brain areas.

These pre- and intra-operative adjuncts included: 1) Direct electrical stimulation (DES); this technique allows neurosurgeons to identify and preserve eloquent brain areas, which are involved in motor, language, memory, and visuospatial functions by electrically stimulating functional brain areas during glioma resection. In order to assess the neurological effect of the electrical stimuli, the patient needs to be awake during this brain mapping procedure that is called the 'awake craniotomy'. 2) Intra-operative Magnetic Resonance Imaging (io-MRI); during surgery, brain shift occurs due to loss of cerebrospinal fluid, edema, and tumor resection. As a result, the neuronavigation system -based on pre-operative MRI images- becomes less reliable during surgery. Io-MRI provides real- time images, without the problem of brainshift, which allows the neurosurgeon to remove residual tumors reliably during surgery. 3) Functional MRI-diffusion tensor imaging (fMRI-DTI) guided neuronavigation; preoperative fMRI of eloquent brain areas and DTI images of white matter tracts (e.g. corticospinal tract) can be integrated into the neuronavigation system, which provides the neurosurgeons with valuable information and an overview of the relationship between the tumor and surrounding anatomic and functional brain areas before and during surgery.

Methods

Electronic medical records were reviewed retrospectively to identify all patients of 18 years or older, who underwent craniotomy for resection of histopathologically confirmed LGGs at the Brigham and Women's Hospital between January 2005 and July 2013. Patients were divided in eight subgroups based on the use of DES, io-MRI and fMRI-DTI guided neuronavigation. Initial and residual tumor were measured volumetrically on pre- and post-operative T2-weighted MR images respectively. The EOR was calculated and the mean EOR was compared between groups.

Figure 1. Box plot showing the differences in mean extent of resection, of tumors located in eloquent brain areas, between subgroups.



Results

Gross total resection (GTR:100% resection) was achieved in 23.4% (30/128 patients included), while in 44.5% tumor resection was > 90% (including GTR cases). Overall, the mean EOR was 81.3% ± 20.5% SD.

Using DES in combination with fMRI-DTI ('DES-FD', mean EOR 86.7% ± 12.4% SD) on eloquent tumors improved the mean EOR significantly after adjustment for potential confounders, when compared with neuronavigation alone ('NN', mean EOR 76.4% ± 25.5% SD, p = 0.001). EOR was significantly less for eloquent tumors in all groups, when compared with non-eloquent tumors in the same group (P<0.001).

Conclusions

Using DES in combination with fMRI and DTI significantly improves EOR when LGGs are located in eloquent areas, compared with craniotomies were only neuronavigation was used. Tumors involving non-eloquent brain areas had a significantly higher EOR when compared with eloquent tumors.

Figure legend

Abbreviations NN: neuronavigation MR: intra-operative magnetic resonance imaging DES: direct electrical stimulation FD: functional magnetic resonance imaging – diffusion tensor imaging guided neuronavigation.

NN: (N=42)
NN-FD: (N=13)
MR: (N=15)
MR-FD: (N=9)
DES: (N=10)
DES-FD: (N=19)
DES-MR: (N=11)
DES-MR-FD: (N=9)

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 section Authors instructions storyline, on the website (www.erasmusmc.nl/erasmusjournalofmedicine) 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 news - Research articles describe one study or analysis, usually from an 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 final or preliminary results of a study. Extended abstracts can concern ongoing research that is not yet published elsewhere which is comparable with a congress presentation thus does not require copyright transfer. An extended abstract can also be submitted after publication in another Journal if possible with extra figures, this does require proper referencing. Number of words: 350 words + 1 figure or table.

Research papers - Here researchers or teachers describe ongoing research projects at the Erasmus Medical centre for which they want to invite students to participate. Number of words: 350.

Systematic reviews - A systematic review is a literature review focused on a research question that tries to identify, appraise, select and synthesize all high quality research evidence relevant to that question in a quantitative way. Systematic reviews of high-quality randomized controlled trials are crucial to evidence-based medicine, and are considered very important by the editorial board of EJM. Besides health interventions, systematic reviews may concern clinical tests, public health interventions, social interventions, adverse effects, and economic evaluations. Number of words: 3000 + 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.

Clinical lesson/question - A clinical lesson should present a scenario and a concrete related question about a disease or condition, the article should elaborate on possible approaches or treatment options for this disease or condition. Conclusion should provice a solid evidence based conclusion on the preferred approach or
treatment. Number of words: 1000 + 1 figure or table.

Case reports - A case report consists of the initial presentation, medical history, examination, tests performed, eventual outcome and discussion on the case backed up by scientific literature. Number of words: 900 + 1 figure or table.

Clinical quiz - A clinical quiz should present a scenario and a concrete related question about the disease or condition, preferably accompanied by a clinical image, and four plausible treatment options or courses of action. Conclusion should elaborate on which is the correct option and why. Number of words: 600 + 1 figure or table.

Clinical images - Clinical images should present a typical abnormality on a photograph/imaging tests of a patient or on an additional investigation. It must be accompanied by an elaboration on the clinical diagnosis. Number of words: 350 + 1 figure. Make sure that the patient is not identifiable or that the data presented traceable to the patient. Additionally, written consent should be obtained from presented patient. We expect the author to refer to scientific literature to back up their case presentations.

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.

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 2007 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.

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[a] Medical students, Erasmus MC University Medical Center Rotterdam, the Netherlands
[b] Dept. of Internal Medicine, Erasmus MC University Medical Center Rotterdam, the Netherlands
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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.

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

The template for authors

Introduction

1. *What is the health-related problem that your research helps to solve?*

2. *What is your strategy to solve the problem?*

3. *What is your research question/hypothesis?*
   Whether a question or a hypothesis, state it in terms of 2 items:
   - variables: the measurable/observable independent and outcome variables that you measured/observed and
   - relationships: the relationships between those variables that your data analyses were designed to determine.

4. *The core concept of the methods you used to answer the research question*
   Briefly describe the core concept of the methods at the end of the Introduction section. This helps readers to understand the complex details that are then presented in the Methods section

Methods section

Organize the details of the Methods section under subheadings. Possible subheadings:

What was studied and study design (subheading)

Describe the details of
- what was studied: sample from a patient/animal population, and
- the design of the study: case-series, cohort study, case-control study, randomized trial, etc.

Data collection (subheading)

Describe the details of how the data was collected/observed

Note
Observable variables will be credible only if qualified observers and validated instruments were used to assess them. Examples of observable variables include patient symptoms, subject responses to open interviews/questionnaires, ultrasound/MRI/CT images, assessments of articles in a literature review etc. In such cases, build credibility in the Methods section; report "who" observed and interpreted the data. For example, "An experienced radiologist interpreted the images."

Note
When reporting on decisions/judgments that were made, use the "we" form—take responsibility for what you did.

Note
The Methods section reports historical facts and must be in past tense.

Data analysis (subheading)

Results section

5. *The core concept of the Results*
   Briefly describe the core concept of the results in a short paragraph at the beginning of the Results section. This helps readers to understand the details that follow. Note just as in the Methods section, this section reports historical facts and must be in past tense.

Then organize the details of your Results under sub-headings, for example:

Patient/animal characteristics
Data
Statistical results

Discussion section

Structure your Discussion to focus on 4 core concepts (6, 7, 8, and 9 below).

6. *The answer to your research question*
   Present this right at the top of the Discussion section—the very first sentence, a present tense statement that expresses—to the best of your knowledge—how the world works as related to your research question/hypothesis. It is a direct answer to the question/hypothesis stated in the Introduction.
7. *Support that answer?*
   a) how your factual findings, (expressed in past tense), support your answer.
   b) relating the findings of others to your answer.
   c) theoretical considerations that support your answer.

Limitations (subheading)

8. *The limitations to that answer*
   Focus explicitly on limitations related to possible confounders:
   - sample size
   - specific locations/medical centers of your study,
   - possible ethnic/cultural variables,
   - uncontrolled patient/subject characteristics and
   - underlying assumptions.

Conclusions (subheading)

The Conclusion is not a summary, but should focus on the consequences of your work. Structure this subsection using separate paragraphs that state 2 main messages (9 and 10)

9. *What are the practical/theoretical consequences of your answer?*
   The value—relevance— of your work: how it helps to solve the problem described at the beginning of the Introduction.
10. *What is a next step to help solve the original problem?*
    - a new research question to be answered
    - a refinement of the present study to reduce limitations
    - a protocol to implement the findings in the clinic

Advice to the reviewers of EJM

For the convenience of our future contributors and our readers, we publish here the advice we give to our reviewers.

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.

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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.

July 2014, Editorial board of Erasmus Journal of Medicine.

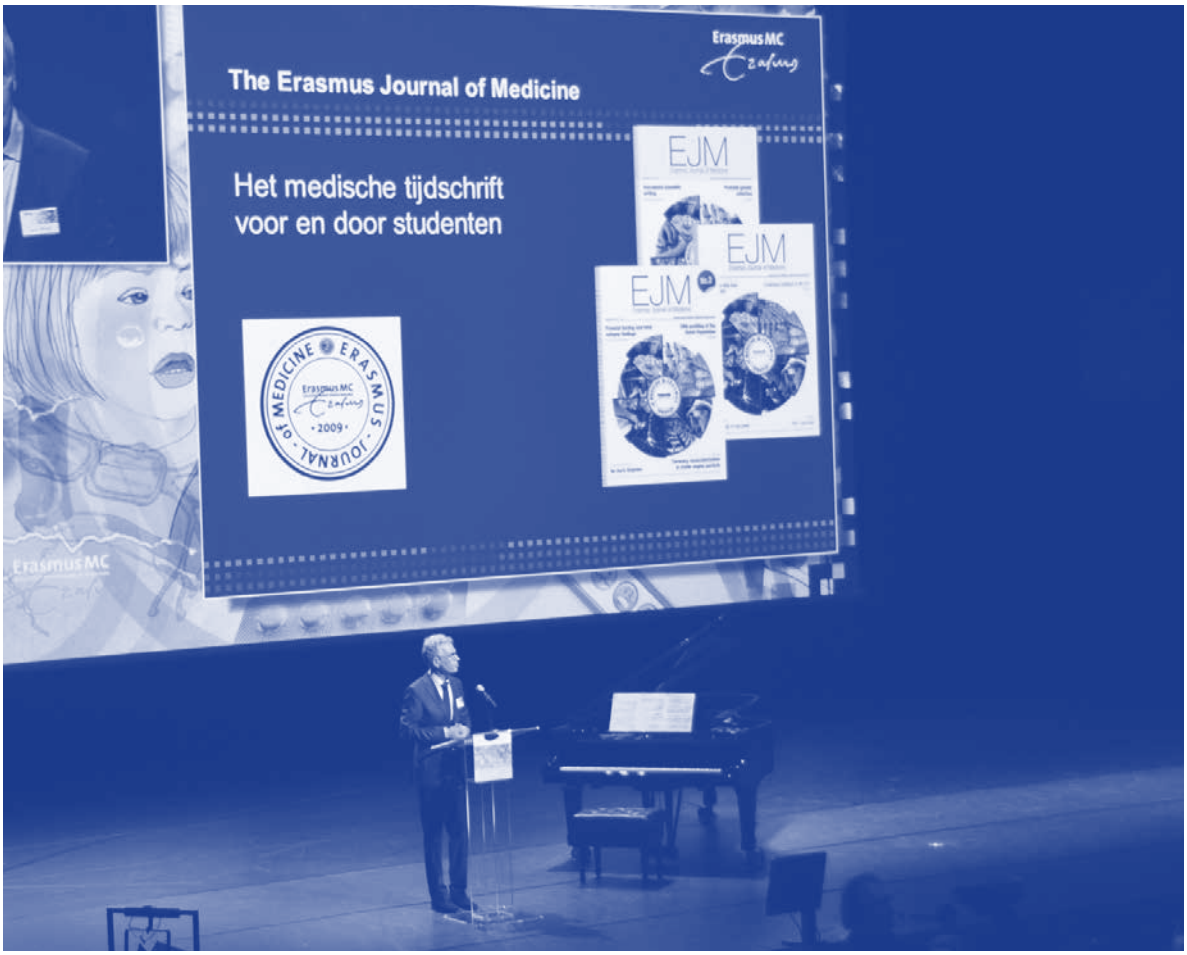
EJM Award

The Erasmus Journal of Medicine promotes the conduct of scientific research by medical and research master students.

Last year, for the first time, the highest rated article was awarded with the Erasmus Journal of Medicine Award during "Lof der Geneeskunst". The award was appointed to Marlene Mende for her pilot study: *Liquid based vs. conventional cytology for evaluation of fine needle aspiration biopsies obtained by pulmonary physicians*. According to the jury: "This study describes how scientific research works in reality. It is not always possible to follow the way as you want, you have to take into account all local protocols and traditions."

The Erasmus Journal of Medicine Award will be assigned again; Lof der Geneeskunst, October 3 2014. A committee composed of experts in the field will select one article that meets the best criteria based on their evaluation for originality, novelty of the idea and the scientific quality.

submit your article: ejm@erasmusmc.nl



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