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Mammographic screening for breast cancer

Systematic review

Treatment of unprotected left main coronary disease

Systematic review

Systematic review

Practical and ethical considerations of cochlear implantation in children

Systematic review

Paracetamol and neurodevelopmental disorders in childhood

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

Top ranked Medicine study

For the fourth year in a row, the study of Medicine at the Erasmus MC, Rotterdam has ranked highest in The Netherlands.[1] Our medical students not only value the high level of education in general, but, in distinction with other universities in our country, in particular the scientific education. Indeed, the curriculum at Erasmus MC is specifically designed to encourage students developing critical thinking skills. In our vision, education, patient care and research form a continuum. We aim to train the next generation of excelling medical teachers, doctors and researchers.

The *Erasmus Journal of Medicine* is a great example of an initiative to familiarize students with medical science. Under supervision by senior researchers, *EJM* is produced for and by students: they write the articles, they are in the lead during the review process and they compose the core of the editorial board. Obviously, they learn quite a bit at all levels, but, more important, they experience the fun of science.

This tenth issue offers a variety of topics, ranging from *perfusion imaging for coronary stenosis detection to prenatal paracetamol exposure and neurodevelopment*. It contains opinion papers, systematic reviews and original research. We hope you will enjoy reading it. Bachelor and Master students at Erasmus MC and other Medicine faculties are herewith cordially invited to submit their work. The essays and reports that they produce to pass their exams are excellent starting documents to become articles that finally end up in *EJM*.

Earlier this year, our colleague dr. Ajda Rowshani stepped aside as Chair of the *EJM* editorial board. We sincerely appreciate all the hard work that she has done to further develop the *Journal*. We will continue in her spirit.

Prof. Jaap Verweij, MD, PhD
Dean and vice-chairman of the Executive Board of Erasmus MC

Prof. Eric Boersma, MSc, PhD, FESC
Chair of the editorial board

1. *http://www.keuzegids.org/ol/gidsen/uni17/1039*

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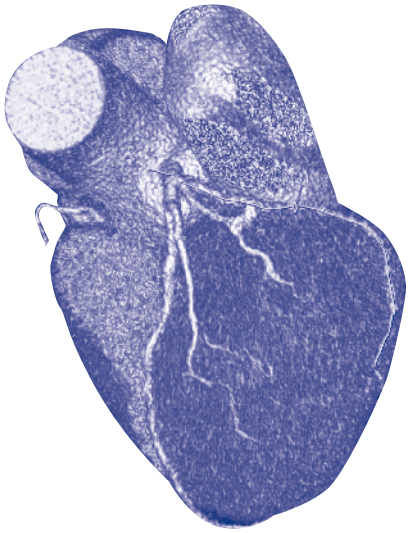

Editorial comment

Surgery or percutaneous treatment of unprotected left main disease: competition or complementary?

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In this issue of the Erasmus Journal of Medicine, a review by Avedissian and colleagues report the outcomes of 4 randomized controlled trials comparing the effect of percutaneous coronary intervention (PCI) using drug-eluting stents (DES) with coronary artery bypass grafting (CABG) in patients with unprotected left main coronary artery disease (ULMCAD). They showed that in patients with ULMCAD and a low or intermediate Syntax score, both PCI and CABG are useful treatment modalities. Yet, in patients with a high Syntax score, CABG should be the first choice of treatment.

Since decades, CABG has been a routine treatment strategy in patients with coronary artery disease as it improves outcomes compared to medical therapy [1]. Grüntzig introduced percutaneous balloon angioplasty, the precursor of PCI with stent, an alternative treatment initially mainly for patients with single-vessel disease and in acute situations such as ST-elevated myocardial infarction [2]. However, treatment of ULMCAD remained a separate case, which was treated with CABG during a long time. This can be explained by the fact that e.g. PCI of ULMCAD is technically challenging due to frequently involvement of the bifurcation, ULMCAD is relatively rare (≈5-7%) in patients with acute coronary syndrome which resulted in a lack of randomized controlled trials and earlier studies showed poor results after intervention (balloon angioplasty) [3-5].

The authors mention that CABG is superior, which is caused by higher rates of revascularization after PCI. Yet, other clinical outcomes of importance such as mortality are comparable for CABG and PCI in patients with low or intermediate Syntax score. The Syntax score is an elegant and useful way to assess complexity of coronary artery disease [6]. However, because of the heterogeneity of the Syntax score between the studies as discussed by the authors, it is difficult to compare results of different studies. In addition, the largest included trial by Morice et al, which is part of the SYNTAX trial, is most discussed in the results section. The SYNTAX trial was initially designed to compare a total population with coronary artery disease, with a subgroup analysis of patients with ULMCAD to test non-inferiority of PCI [5]. As mentioned by Teirstein et al. these results are therefore more or less observational findings, but the authors of the current review fail to mention this important context [7].

Nonetheless, the findings of the reviewers are in line with the European guidelines of 2014 on myocardial revascularization, which may be expected as similar studies were compared. Based on these studies and consequently the corresponding guidelines, PCI has increasingly been performed over the years [8]. The time-period selected by the reviewers did not include novel randomized controlled trials focusing on ULMCAD and either CABG or PCI with DES compared to the guidelines. It is worth mentioning that recently, the large multicenter international EXCEL trial has been published, including nearly 2000 patients with ULMCAD assigned for either PCI with second generation DES or CABG [9]. The authors concluded that both PCI with DES and CABG are useful treatment strategies when performed by an experienced team. The outcome of primary end-points including death stroke and myocardial infarction in patients with low and intermediate Syntax score was non-inferior for PCI compared with CABG after a 3-year follow-up. This is, again, in line with previous reports. It is expected that a longer follow-up is of this trial to compare results between both groups will be performed. Finally, the effect of PCI with DES in patients with a high Syntax score remains unknown and might still be of interest for future studies.

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Can invasive coronary angiography be replaced by non-invasive CT coronary angiography?

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The meta-analysis of van der Wel et al., in addition to a systematic review, addresses one of the key problems of detecting coronary lesions.[1] Today, the golden standard is invasive coronary angiography (CAG). During a CAG procedure a thin, flexible catheter is advanced via the femoral or radial artery into the coronary arteries. By using X-rays the inside of the coronary arteries can then be inspected. In this way coronary lesions can be detected. The golden standard for defining hemodynamically significant stenoses is the CAG-based Fractional Flow reserve (FFR). Lesions with low FFR values are candidates for revascularization treatment. Although cardiac catheterization rarely causes serious complications, it is a serious and extensive investigation.

Attempts have been made to develop more patient-friendly, non-invasive alternatives. One of these modalities is Computer Tomography (CT). CT coronary angiography (CTA), using scanners with at least 64 slices, can be used to rule out obstructive coronary stenosis. Thus, inappropriate invasive CAG can be avoided in patients with negative CTA, although CTA was not a replacement for CAG. However, a few years ago, a new modality, Computer Tomography Myocardial Perfusion Imaging (CT-MPI) was published by Rossi et al., who reported that CT-MPI might result in a reduction of invasive CAGs.[2]

However, CT-MPI had to be verified against the gold standard FFR. The present meta-analysis found 4 (European) studies that compared both modalities. The studies had small sample size, so that, taken singly, these produce inconclusive results. By combining all available information the meta-analysis aims to overcome this drawback. Altogether, the studies enrolled 210 patients and 600 coronary arteries. Using the FFR as the golden standard, CT-MPI had a sensitivity of 89% and a specificity of 88% to diagnose hemodynamically significant stenoses. The diagnostic accuracy was 88%. These are promising results and may help the field to improve the non-invasive CT-MPI to replace CAG. Nevertheless, as the authors appropriately address, the combined sample size was still low and the 4 studies included in the meta-analysis were too heterogenous to draw final conclusions. A future large multicenter study should be carried out to investigate whether CT-MPI can replace CAG-FFR.

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Editorial comment

An effort to interpret the literature on safety of paracetamol use during pregnancy may provoke a headache

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Paracetamol is one of the most frequently used over-the-counter analgesic and antipyretic drugs. It is considered as a safe drug and self-medication is common also during pregnancy [1]. However, the thalidomide (or Softenon) tragedy has taught us that even drugs that are initially considered safe can lead to adverse effects when used during pregnancy [2]. The problem is that not every adverse drug effect leads to the type of extensive phenotypical changes as thalidomide does. So how can we identify other relevant drug adversities that are not so easily spotted?
When it comes to drug-related adversities, it is important to realize that the vast majority of our knowledge on the teratogenicity of drugs come from studies in animals and observational studies in humans since it is unethical to perform phase I or II studies in pregnant women and most clinical trials exclude pregnant women [3]. Animal studies have indicated that prenatal paracetamol exposure may affect brain derived neurotrophic factor (BDNF; a regulator of neuronal cell migration, axonal/dendritic branching and synaptogenesis), serotonin (important for maturation of the brain and neuroplasticity), cognitive function, behavior and working memory [4-6]. Such alterations may seem mild but it is important to realize that pregnancy is a vulnerable period during which already small changes can affect the development of the child and consequently its health during adulthood (fetal programming).

Only recently, the potential link between maternal paracetamol use during pregnancy and adverse neurodevelopmental outcomes in the offspring . In the current study, Afadass, Soares and El Marroun provide a literature overview of studies investigating this link. Taken together, the six studies that they identified showed that maternal paracetamol use was associated with offspring ADHD symptomatology, autism, gross motor and communication development but not IQ. Upon the authors discussion of the strengths and limitations of each study it becomes quite apparent that there are many caveats that one should be aware of when interpreting the current body of evidence in this field. The fact that this is quite a novel field of research is reflected by the fact that many studies did not have data available on the dosage of paracetamol used. The ability to investigate a dose-dependent effect would be very valuable and a study that would have the initial aim to investigate the association of paracetamol use and offspring neurodevelopment would have collected this data.
The authors also mention confounding by indication, meaning that women who have an indication to use paracetamol (i.e. because of fever, headache) may have an underlying condition (or a more severe form), for example an infection. Consequently, it could appear that paracetamol use is associated with adverse neurodevelopment outcomes while the true risk increase is caused by the infection. Something that the authors do not mention, but that is also of relevance for this paper, is publication bias. Five out of six studies had a positive finding, and the only negative study was published more than 26 years before the positive studies. This raises the question whether no other studies have been performed in the meanwhile, or whether they were negative and therefore ended up not being published (for example because the authors rather publish something else or because journals did not want to publish it). Also residual confounding is mentioned, meaning that there could still be confounding present because variables that confound the association are unknown and/or unmeasured. In this respect, it is interesting to note that only recently, a study from the UK was published on the same topic [7]. In this study, the authors show that the association of paracetamol use in pregnancy with child behavioral symptoms did not change after additional adjustment for postnatal paracetamol use of the mother, paracetamol use of the father or the presence of genes of the mother that are associated with ADHD [7].
These type of phase IV studies, in which adverse drug outcomes are found after the drug has reached the market and a large number of individuals start using them, are important because they allow us to identify previously unknown adverse drug effects. Paracetamol use in pregnancy is common, and already small changes in neurocognitive potential can have large effects on a population scale. This is illustrated by studies that for example show that already small changes in neurobehavioral disease due to exposure to endocrine disrupting chemicals (such as pesticides and plasticizers) in Europe costs more than €150 billion a year [8]. Given the large public health indications of this potential association, further studies will be needed to define if maternal paracetamol use leads to suboptimal child neurodevelopment.

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Systematic Review

Mammographic screening for breast cancer: an ounce of prevention worth a pound of harm?

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Introduction

Although breast cancer screening by means of mammography was implemented almost 20 years ago it is still a subject of much controversy. Recent systematic reviews suggest a significant reduction in breast cancer related mortality due to screening. There are however reasons for skepticism. The incidence of advanced breast cancer has not decreased. Apart from the dubious benefits of screening, a myriad of adverse effects has been reported. Overdiagnosis results in unnecessary mastectomies, lumpectomies, chemotherapy and radiotherapy which all cause undue physical harm and squander of resources and specialist care. False-positive mammograms cause unnecessary psychological distress. In scientific literature, particularly by interested parties, these drawbacks tend to be underexposed. From an ethical point of view there are firm objections to breast cancer screening in its current mode. To live up to the principle of beneficence the principle of non-maleficence should not be neglected. Recent evidence suggests an alternative way of screening in which adverse effects are diminished. This paper looks to explore whether mammographic screening is still the best approach in view of the above points and to contrive the answer to a question that affects hundreds of thousands of women: 'Should mammographic screening be reconsidered?'

Screening for hundreds of thousands of women

Every year hundreds of thousands of women in the Netherlands receive an invitation to participate in the population screening program for breast cancer. Since 1996, women between the ages of 50 to 69 years of age have the opportunity to be screened for malignancies in the breast. In 1998, women 70 to 75 years of age were added to the population that is invited to be screened every other year[1]. Participating women are subjected to mammography. The mammograms are subsequently examined by two independent radiologists. Outcomes are measured against the BI-RADS (Breast Imaging Reporting and Data System) classification. The main goal of this biennial screening is early detection of breast cancer, which is the most common form of malignancy in women[2]. Early detection, before the cancer has metastasized, can make the difference between a curative therapy or a palliative treatment[3]. Between 1990 and 2012 the breast cancer screening program has been evaluated by a national team of scientists from the Erasmus MC and the Radboud University Medical Centre. This National Evaluation Team for Breast cancer screening (NETB) estimated the number of deaths prevented annually by the breast cancer screening program to be 775[4]. Notwithstanding this report and other studies from Dutch soil that affirm the benefit of mammographic screening[5,6], disagreement on this topic persists in the scientific community. Contradictory research results, mostly of foreign origin, and polemic within the scientific realm concerning the benefits and drawbacks of mammographic screening, result in conflicting media coverage[7,8]. There has been a measurable decline in participation since 2007 which is potentially the consequence of this. It lies in the interest of all Dutch women that a clear answer is contrived to the question: 'Should the population screening for breast cancer be reconsidered due to recent scientific insights or not? Do the benefits still outweigh the risks?'

Reduction in breast cancer related mortality

The most important pillar that supports the national breast cancer screening program is the axiom that breast cancer screening reduces breast cancer related mortality. There is no doubt that the 5-year survival of breast cancer patients has increased and mortality has decreased significantly since the onset of the breast cancer screening[2]. Needless to say, improved treatment contributed vastly to these developments2. The exact numbers that can be attributed to screening is hard to quantify. In recent years a number of studies have been published that sum up the results of all relevant randomised controlled trials that have been conducted. These systematic reviews suggest a 15% to 20% reduction in relative risk of breast cancer related mortality for women participating in the breast cancer screening compared to women not participating[9,10]. These reviews originated in the United States and the United Kingdom respectively, both countries with a similar breast cancer screening program. This makes the results from these studies applicable for the Dutch population. With this in mind, these figures seem to be a major justification for continuation of the breast cancer screening program.

There are however some matters that raise doubts about this apparent success. First of all, remarks can be made about the U.S. Preventive Services Task Force review and the Independent UK Panel on Breast Cancer Screening review concerning methodology. It appears that in some of the trials that were included in these systematic reviews the randomisation process, when subjected to the criteria of the Cochrane Handbook, should have been considered suboptimal[11,12]. When data is analysed from solely the trials with an optimal randomisation process little remains of the beneficial effect of mammographic screening on breast cancer related mortality[11]. Second is the fact that the current course of epidemiology is not consequent with a successful screening program. In countries with an established breast cancer screening, at best only a marginal decrease in advanced breast cancer has been observed[13,14]. This fact undermines the efficacy of breast cancer screening, for a decrease in advanced cancer has always been regarded as an early indication of success[15]. An example of this is the decreased incidence of cervical cancer after the implementation of the pap smear[2].

The drawbacks of mammographic screening

As stated earlier it is unclear the extent in which mammographic screening reduces breast cancer related mortality. Doubts raised by this obscurity are reinforced by the adverse effects that screening has. Screening on a large scale by means of mammography in most cases detects benign abnormalities. Additionally malignancies are discovered that would not have caused harm to the individuals within their lifetime[11,14,16]. For example, carcinoma in situ which in many cases does not metastasise, is found more often by screening than on the basis of clinical symptoms[17]. In women 39 to 74 years of age this overdiagnosis accounts for 30% of screen detected breast cancers[11,14,18]. In women 40 to 59 years of age, 22% of screen detected cancers is due to overdiagnosis[19]. Healthy women are being labelled and treated as if they were breast cancer patients. These women are subjected to surgery, chemotherapy and radiotherapy while they never would have been diagnosed with breast cancer had they not participated in mammographic screening. Besides the psychological distress these women are exposed to, for instance anxiety and depression, they experience all kinds of side effects due to therapy. Lymphedema, fatigue, sexual dysfunction, vasomotor symptoms and cognitive complaints are among the most common[20]. At the same time there is an increasing amount of false positive mammograms. The estimated risk of a mammogram being erroneously labelled positive for women loyally attending biennial screening accumulates to 16%[6]. A false positive mammogram is associated with anxiety, insomnia and a negative impact on sexuality and relations with friends and family[21]. Complaints due to a false positive mammogram can persist long after cancer has been excluded[22]. Apart from unnecessary harm these false positive mammograms and overdiagnosis result in squander of funds and specialist care. Scientists and authors who have an interest in large scale mammographic screening tend to ignore, play down or disclaim these drawbacks[23,24]. Lastly there is a theoretical risk of creating a false sense of security. In the case of an aggressive tumour the interval between two mammograms is long enough for the tumour to metastasise[16]. These interval tumours are usually of a more fatal nature than screen detected tumours[25]. A large scale mammographic screening potentially creates a certain sense of safety causing participating women to underestimate the chance of interval cancer. A possible consequence of this could be that participating women are less attentive of abnormalities in the breast.

Proportionality and subsidiarity

The continuation or discontinuation of the breast cancer screening program in the current modus, by means of mammography, is not only a question of medical science but also one of medical ethics. The idea of preventive screening, to avert morbidity and mortality, is derived from the ethical principal of 'beneficence'. An important underlying idea behind screening however is also that the population should not be needlessly exposed to risks[26]. This idea originates in the ethical principal of 'non maleficence'. Screening of healthy individuals results in short as well as long term psychological damage due to false positive mammograms[21,22]. In addition, abnormalities are being detected and treated which would, in the absence of screening, not have resulted in illness[10,11,14]. This results in harm due to all kinds of unnecessary treatment such as surgery, chemotherapy and radiotherapy[20]. The ultimate question in this matter is: Is the amount of harm caused by mammographic screening proportionate with the reduction in mortality; Is the breast cancer screening program in accordance with the ethical principle of proportionality?

Assuming a reduction in breast cancer related mortality of 15%, it can be stated that for every 2000 women that participate in mammographic screening during 10 years one life is saved[9,11]. Taking in account an overdiagnosis of 30%, in this group 10 women receive a redundant cancer diagnosis and are unnecessarily treated[11,14,18]. Allowing for a false positive recall rate accumulating to 16% in 25 years, hundreds of these women will have a false positive mammogram[6,11]. Apart from the principle of proportionality, medical ethics reckons with the principle of subsidiarity. It implies that when an intervention is needed, the least harmful option has to be employed. A recent Canadian study in which annual mammographic screening was compared with annual physical examination by trained nurses placed subsidiarity of screening by means of mammography in an entirely different context. After a 25 year follow-up, breast cancer related mortality was observed to be equivalent whereas in the mammography group a 22% breast cancer excess was found due to overdiagnosis[19]. These results support the conclusion of an earlier article form Canadian soil that stated that mammography makes no contribution to the benefit of screening by annual clinical breast examination[27]. An explanation for these observations might be found in the fact that mammography can detect cancer in a non-palpable stage. As mentioned earlier however, some tumours do not cause morbidity and mortality within the patient's lifetime. It might be assumed that the bulk of these tumours are non-palpable. In the Canadian National Breast Screening Study half of screen detected non-palpable cancers were over-diagnosed. Physical breast examination self-evidently does not detect non-palpable tumours and the fact that cancer is detected only when it has grown to a palpable size apparently does not lead to an increased mortality[19].

To prevent or to cure?

Recent scientific observations indicate that the breast cancer screening program by means of mammography requires a thorough reconsideration. The actual absolute reduction in breast cancer that can be attributed to screening is still subject to uncertainty. The most solid evidence, coming from the systematic reviews of the US Preventive Services Task Force, the Independent UK Panel on Breast Cancer Screening and the Cochrane Collaboration, presents percentages of approximately 15% - 20%. There are however reasons to pose a skeptical attitude towards these figures. In addition to a decrease in advanced breast cancer in the overall population, one would expect to find decrease in incidence of advanced breast cancer when screening is implemented. This however is not the case. Apart from a lack of solid evidence to confirm the efficacy of breast cancer screening, an abundance of adverse effects has been observed due to mammography. Overdiagnosis results in unnecessary mastectomies, lumpectomies, chemotherapy and radiotherapy which all cause undue physical harm and squander of resources and specialist care. False-positive mammograms cause unnecessary psychological distress. In scientific literature, particularly by interested parties, these drawbacks tend to be underexposed. Finally there is the calming effect and a false reassurance that could possibly arise from large scale screening whereas in reality it offers no protection from aggressive tumours. The benefits women could possibly gain from participation in mammographic screening are in no way commensurate with the risks they are exposed to. The ethical principle of 'non maleficence' is being trampled in order to save a limited number of lives, and that is not in accordance with the underlying ideas of preventive screening.

Conclusion

Breast cancer is a prominent cause of morbidity and mortality in women and therefore awareness of- and research on this topic is of great importance. The recently published twenty five year follow-up of the Canadian National Breast Screening Study might offer an alternative way of conducting breast cancer screening. Physical examination by trained nurses instead of mammography could turn out to be equally effective and prevent the better part of adverse effects. Although this finding indicates a possible way to get rid of overdiagnosis and undue harm, most investigations into the efficacy of breast cancer screening have compared mammography to no screening at all. Inquiry should be made specifically into the effect of mammography compared to the effect of physical breast examination on long term breast cancer mortality. A frequently quoted statement of Founding Father Benjamin Franklin is that 'An ounce of prevention is worth a pound of cure.' Prevention by means of mammography however has so far not yielded satisfactory results. As long as this stays unaltered, directing all efforts on the treatment of breast cancer seems an advisable approach. Recent successes such as hormonal therapy and immunotherapy are indications of the progress that ,with resources now being spent on screening, could be achieved in the field of pharmacotherapy.

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Systematic Review

"When parents turn a deaf ear"

Practical and ethical considerations of cochlear implantation in children

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Acknowledgments

Summary
315 children with permanent hearing loss were born in the Netherlands in 2013.Deaf and hard of hearing children can be implanted with a cochlear implant (CI). A CI allows them to hear better, to understand speech and sometimes even produce speech themselves.

Many parents of deaf children get their child implanted with a CI as soon as possible. However, some parents don't want their children to receive a CI. Some of these parents say that deaf culture will be lost and that deafness is not a handicap. However, the author raises some objections against these arguments. Deaf culture will not disappear, because some people are not eligible for a CI. Furthermore, it is not fair to deny children a change at an ordinary development only to preserve the culture. Deafness is a handicap, since they are dependent on assistance to realize their full potential. Another argument from the deaf community is fear of complications. CI implantation is a safe operation.

An ethical dilemma now arises between the principle of respecting autonomy and the principle of beneficence. Even though a CI is effective, the benefits for the child do not outweigh the infringement on the freedom of choice of the parents. Therefore, a medical doctor should not implant a CI without permission. They should, however, do everything in their power to convince deaf parents of the benefits of a CI.

Introduction

In 2013, 315 children were born with permanent hearing loss. The total number of live births in that same year was 171.341 [1]. This amounts to an incidence of permanent hearing loss of 1.8 per 1000 new-borns. Nowadays, congenital deaf children are able to learn spoken language when they receive a cochlear implant (CI). A CI allows deaf children to hear better, to understand speech and sometimes even produce speech themselves. This is because a CI translates soundwaves into neuronal stimuli that directly stimulate the auditory nerve. This mimics the normal hearing pathway[2].

Many parents of deaf children see this new development as a miracle and get their deaf child implanted with a CI as soon as possible. There are some parents, however, that don't want their children to receive a CI[3]. Do these parents have the right to refuse treatment for their children? Or are doctors allowed to implant a CI in deaf children, even without their parents' consent?

The Cochlear Implant: A solution for deafness and heard of hearing

Deafness at a young age can have a negative impact on spoken language development, social and emotional development and general education[4]. Hearing aids or a cochlear implant can be used to combat deafness. Early detection and treatment provide better language development[2,5].

The severity of deafness is one of the key determinants in choosing the treatment.CI is the standard treatment for children with severe hearing loss[5,6]. Better hearing, better development of speech, better development of spoken language, better educational achievements, higher quality of life and a higher chance of gaining employment are all advantages of a cochlear implant[2,4,7].

A cochlear implant is implanted during surgery. In 2.3% of all cases, major complications arise and in 16% of all cases there are minor complications. Minor complications are mostly temporary weakness of the facialis nerve, acute ostitis media or wound infection. Major complications are cholesteatoma, persistent wound infections or eardrum rupture[8]. A cochlear implant is considered reasonably safe. Routine hospital stay after CI implantation with no complication is 24 hours[8,9].

Cochlear implants work by stimulating the auditory nerve fibres. In a person with normal hearing soundwaves travel into ear canal and reach, and vibrations reach, through the bones the mid-ear, the cochlea. The sensory hair cells within the cochlea transform these waves into a neural signal. This signal is transmitted via the cochlear nerve to the auditory cortex. CIs work by substituting the sensory hair cells with electrodes that stimulate the auditory nerve fibres directly. CI has two components; an external component, worn behind the ear and an internal component, which is surgically embedded in the mastoid. A CI is effective in a wide range of hearing problems, from genetic causes to cochlea dysfunction after infection. It is effective in all sensorineural types of hearing loss, provided that central auditory pathways and the cochlear nerves are intact. By stimulating the auditory nerve, a CI allows deaf children to hear

better, which helps them comprehend spoken language. This higher comprehension is beneficial to the expression of spoken language[2].

A CI is not effective when the cochlear nerve is not developed, deafness is due to lesions of the central auditory pathway. Implantation of a CI is not possible when there is massive cochlear ossification that prevents electrode insertion[4].

Why do deaf parents refuse a cochlear implant for their child?

Occasionally, parents who refuse the CI are deaf themselves. Some of them are afraid that deaf culture will be lost if all deaf children will receive a CI[3]. Some deaf parents appreciate their culture and claim that sign language and deaf culture should be protected[10]. They invoke a treaty of the United Nations, intended to protect minority languages and cultures[11]. However, some objections can be raised against this argument regarding culture. Firstly, deaf culture will not disappear that quickly. A deaf child with a CI will still have to learn sign language to communicate with their deaf parents. There are also children and adults who are not eligible for a CI. They will continue to use sign language. Likewise, children who do not respond well to a CI will also continue to use sign language.

Another objection to this argument is that it is not fair to deny a child the chance at an ordinary development, only to conserve a culture. For some deaf persons, deaf culture has an intrinsic value[10]. But does this value outweigh the well-being of a child? The author believes that the health and well-being of a child should prevail above preservation of a culture.

A different argument that is brought forth by some of the parents who refuse a CI is that deafness is not a handicap and that therefore a deaf person does not need to be 'fixed'[3]. They deem that deaf children should not have to undergo an invasive operation, because without it, children would continue to live their lives as a fully functional part of the deaf community. However, much is to be said against this argument. Fact is, deaf people have lost one of the five important senses. They are dependent on assistance to realise their full potential. Some deaf children go to special schools. Most of the deaf people need translators when communicating with other people such as salespersons, teachers and doctors. Deaf people can function in society but only because society provides special features, such as translators, TV-news reports with sign language interpreters and special schools[12]. Therefore, the author feels that it is wrong to deny that deafness is a handicap. Because a person can function in society with assistance does not undermine the fact that the person is still disabled. An easy example: nobody would argue that a double amputee is not disabled, just because they use their wheelchair and because they can function in society.

Another argument from parents is that they are afraid of the complications that are possible with a CI implantation. However, as stated before, CI implantation is a relatively safe operation[8,9,13].

Before it's too late

It is important to implant the cochlear implant at a very young age to get the best results. Studies show that children, who receive a CI prior to age two, perform significantly better than those who receive a CI at a later age in vocabulary, speech intelligibility, general language ability and phonological processing skills[14,15]. This is due to the fact that during the first three years of life the brain is able to create new neural connections after receiving auditory stimuli. After three years the brain loses this feature[13,16]. Children between ages of 11 and 14, who have been deaf their whole lives and receive a CI, often can't speak or understand spoken language, even after years of CI use[14].

This is one of the main problems of this ethical issue. If the parents decide not to implant their child with a CI, and the child disagrees at a later age and then sets out to receive a CI, the CI is not as beneficial as it could have been[17]. The parents' decision is an irreversible decision.

Ethical considerations

An ethical dilemma arises between the principle of respecting autonomy and the principle of beneficence. A physician will want to respect the autonomy of the patient, or in the case where the child is under 12, the autonomy of the patients' parents. Respecting the autonomy of the patients' parents can mean not implanting a CI. On the other hand, a physician will want to implant a CI because it is very beneficial for children who are eligible for CI implantation.

Indeed, the benefits for the child are fairly large. However, since a deaf child is able to communicate through sign language and can, with the right assistance, function in our society, there is no harm done to the child by not implanting it by a CI. When a child is not offered sign language, actual harm, by communication deprivation, is present. If you prevent harm, you are more easily allowed to infringe upon the autonomy of the parents. However, if an intervention does not prevent harm but improves life, there are several conditions before you can intervene in the freedom of choice.

Firstly, the intervention has to be effective[18]. The CI is a very effective treatment for the deaf and the hard of hearing, in whom a CI is indicated[5]. Furthermore, the infringement of the freedom of choice has to be as small as possible[18]. The implantation of a CI is a big infringement of the freedom of choice of the parents'. Their child's life will be rather different in regards to education, hobbies and job prospects. A CI is, as said before, not without complications[8]. What would happen if the parents are forced to have their child implanted with a CI and there is a major complication? What should be done when a child dies because it developed meningitis after surgery? No meningitis has been reported in recent studies in CI implantation in children. However, from the adult population we know that meningitis can occur after implantation of CI[19].

Finally other factors need to be taken into account[18]. The author believes that if deaf parents are forced by the medical world to give their child a CI, this could lead to a strained relationship between the deaf community and the medical community. Therefore, parents will be less likely to contact the medical community.

To make an informed decision and to get a better insight into these principles, other similar ethical dilemmas should be looked into[20]. An example in which the wishes of the parents are not respected is in the case of a lifesaving blood transfusion

in children of Jehovah Witnesses. If the child is under 18, the doctor will give the child a life-saving blood transfusion, even if the parents will not allow it. In this case the life of the child is held in higher respect than the autonomy of the parent[21].

Another, often debated, example is home environment and asthma. There are children with severe asthma, who live in an environment which is not suitable for a child with asthma, because of smoking or pets. These children can live with their asthma, however this environment will cause more frequent exercabations and they will require more treatment, compared to when they would live in a clean home. Regarding the wellbeing of the child, the child would be better off to living in a smoke-free and pet-free house. This would improve their live. However, in the Netherlands, a physician is not allowed to remove a child from their environment, unless the child is in real, life-threatening danger. In this case the autonomy of the parent is held in higher respect then the well-being of the child[22].

Autonomy of the patient is a right that is regarded as one of the most important rights. At its root lies freedom: freedom to be free of force or harm and to be free to make your own choices. This freedom is one of the most basic rules of our society. This freedom allows people to make decisions about their life and live in the way that they see fit, as long as they don't infringe upon the rights of other people. This freedom has to be respected by health care workers. This freedom can only be infringed upon if the circumstances are very severe: your own life of somebody else's is directly in danger or there is very serious harm.

When there is infringement upon patient autonomy in other, non- life threatening instances, it becomes very complicated rather quickly. Are we going to forcefully administer medication to people who don't want to take their medicine, in their own best interest? Are we going to force obese patients to run on a treadmill? How is the force administered, because that force can also harm the patient.

Another problem with infringing upon autonomy is that there is sometimes not a clear cut solution to the problem. Some studies suggest that there is a beneficial effect of 2 glasses of alcohol each day. Other studies dispute this. Will it be made mandatory to drink 2 glasses of alcohol each day, only to be discovered later that drinking no alcohol is actually more beneficial?

Authors' opinion

To combat these problems with autonomy versus beneficence, a clear cut distinction should be made: patients' autonomy can only be infringed upon if there is a life-threatening situation or serious harm .

The case of a deaf child is most similar to the case of home environment. Deafness is not an acute, life threatening condition. Therefore it is important to treat it the same way you would treat a case of suboptimal home environment. Both implanting a CI and removing the suboptimal environment would improve the life of the child. A physician should try to improve the situation and convince the parents, but no force should be used. A CI should not be implemented against the wishes of the parents. Even though a CI is highly effective, the benefits for the child do not outweigh the infringement on the freedom of choice of the parents. Additionally, the relationship between the deaf and the medical community should not be strained further.

Therefore, a medical doctor should not implant a CI without permission. They should, however, do everything in their power to convince deaf parents of the benefits of a CI. The higher quality of life, better integration into regular education, higher chances at a job and the fact that deaf culture will not perish are all very important arguments to help convince the parents of the benefits of a CI. If the parents cannot be persuaded, it is rather regrettable, but the doctor cannot implement the CI.

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Systematic Review

Is it Clinically Relevant to Perform a protocol MAG3 Scan Postoperative?

A Retrospective Monocentric Study of 402 Cases.

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Abstract

Objective: **A MAG3 scan can aid in renal transplant patient care, especially when allograft dysfunction develops. However, the value of a protocol MAG3 scan is unknown. Therefore, we evaluated the clinical relevance of a protocol MAG3 scan one day after transplantation.**

Methods: **We performed a retrospective, single-center, case study including all patients who underwent renal transplantation between January 2013 and January 2015 at the Erasmus Medical Center Rotterdam.**

Results: **In our cohort of 396 patients, 177 patients had an abnormal MAG3 scan. In 31 patients the result of the MAG3 scan led to a change in clinical care, including a change in medication in 5 patients, further diagnostic test in 17 patients and surgery in 6 patients. Patients with an abnormal MAG3 scan had significantly older donors (58.0 yrs; range 0-86 yrs vs 51.5 yrs; range 14-89 yrs, p<0.01) more often a postmortal donor (58.7% vs. 11.8%; p<0.01) and a longer warm ischemia time (22 min; range 8-52 min. vs 19 min; range 10-58 min, p<0.01). Despite these significant differences, considerable overlap existed between these characteristics. The protocol MAG3 scan contributed to the diagnosis in 1 of the 3 patients with an urinary leakage. Moreover, in 3 of the 5 patients with a kidney infarct on protocol MAG3 scan, additional testing ruled out a kidney infarct.**

Conclusion: **The protocol MAG3 scan led to a change in renal transplant patient care in 31 of 396 patients. Despite significant and independent differences in donor age, donor type and warm ischemia time between patients with a normal or abnormal MAG3 scan, no cut-off point in these characteristics could be found to limit this per protocol strategy. Since a MAG3 scan is not only costly but also a burden for patient and nurses, our data suggest that the role for a protocol MAG3 scan in renal transplant patient care is very limited.**

Introduction

Renal transplantation is the treatment of choice for patients with end-stage renal disease. The donor kidney is placed extraperitoneally in the patient's iliac fossa, with end-to-side anastomosis to external or internal iliac vasculature [1]. A widely used tool to evaluate postoperative kidney dysfunction, is a MAG3 scan. This renography is a simple and non-invasive method for the evaluation of renal transplants, using the radiopharmaceutical mercaptoacetyltriglycine (Tc99m-MAG3)[2]. This radiochemical provides a low radiation dose, good image quality with low background activity and excellent first-pass characteristics [3]. After intravenous injection, mercaptoacetyltriglycine is only taken up by the tubuli of the kidney, where it accumulates and subsequently is excreted in the urine [4]. In this way perfusion, extraction and excretion as well urinary obstruction or leakage can be detected [5,6]. Previous studies of MAG3 scans performed shortly after transplantation showed the ability to predict the long term graft outcome, with a high sensitivity, but with low specificity [4,7]. However, the predictive value of the MAG3 scan was significantly lower than that of the serum creatinine value, thereby greatly limiting its use. The Erasmus Medical Center Rotterdam also performs a MAG3 scan on every post renal transplantation patient, usually one day after transplantation. In this report we evaluate whether this strategy is clinically relevant and influences patient management in the early post-operative period.

Methods

Study design and patients

We performed a retrospective, single-center, case study. In this study, patients who underwent a renal transplantation between 1 January 2013 and 1 January 2015 at Erasmus Medical Center Rotterdam in The Netherlands were included. Patients were excluded if a protocol MAG3 scan was not performed within 4 days after transplantation.

Clinical outcome
MAG3 scans were routinely evaluated by different nuclear radiologists. Based on their report we scored the results as normal, urinary leakage, obstruction, decreased extraction, decreased excretion, decreased perfusion and/or infarction or combined abnormalities. If the MAG3 scan was reported as abnormal, we searched the medical charts for any effect on patient care, within 4 weeks after the MAG3 scan was performed. We divided the effect on patient care in to 4 categories; a change in medication, further diagnostic tests and (not) performing an operation or procedure. When multiple MAG3 scans were performed, only the protocol scan was used to determine the clinical outcome.

Statistical analysis
We performed overall group comparison on the categorical variable using Pearson chi-square test. For continuous variables we used either an unpaired T test (normally distributed data) or Mann-Whitney U test (not-normally distributed data). All statistical tests were two-sided and $p < 0.05$ was considered as statistical significant. We analyzed these results using IBM SPSS Statistics, version 23.

Results

Between January 2013 and January 2015, 402 patients underwent renal transplantation. 6 patients did not have a MAG3 scan performed within 4 days and were excluded. Therefore 396 patients were analyzed. (figure 1 and table 1)

Normal and abnormal MAG3 scan
The MAG3 scan was performed after a mean of 1.4 ± 0.04 days after kidney transplantation. In 177 of the 396 patients, an abnormal MAG3 scan was seen (44.7%). The abnormalities consisted of urinary leakage in 3 patients (0.7%), an obstruction in 15 patients (3.7%), a decrease in extraction in 83 patients (20.6%), a decrease in excretion in 111 patients (27.6%), a decrease in perfusion in 24 patients (6.0%) and/or a renal infarct in 5 patients (1.2%). 57 patients had a combination of these abnormalities.

Influence on clinical decision making
177 patients had an abnormal MAG3 scan and in 31 (17.5%) of these patients, this influenced their treatment. The characteristics of these patients are shown in table 3. In 5 patients, the result of the MAG3 scan led to a change in medication, in 17 patients to further diagnostic research (echography, MAG3, SPECT-CT and/or biopsy) and 6 patients to surgery, including transplantectomy, inserting a nephrostomia catheter and replacing or flushing the splint or transurethral catheter. In 3 patients a combination of interventions was performed.

Characteristics of patients with an abnormal MAG3 scan
In patients with an abnormal MAG3 scan, the donor was significantly older (58.0 yrs; range 0-86 yrs vs 51.5 yrs; range 14-89 yrs, $p<0.01$).
Moreover, the percentage of postmortal donors was significantly higher in the case of an abnormal MAG3 scan (11.8% vs 58.7%; $p<0.01$) as was the warm ischemia time (22 min; range 8-52 min. vs 19 min; range 10-58 min, $p<0.01$) (table 2). These characteristics were independently associated with the MAG-3 scan outcome (all $p<0.05$ in a multivariate regression model).
Patients, whose abnormal MAG3 scan influenced the clinical outcome (n=31), also had a significantly higher warm ischemia time (21 min; range 13-50 min. vs 20 min; range 8-58 min, $p=0.026$). (table 3)

Figure 1- Flowchart of the patients included in this study

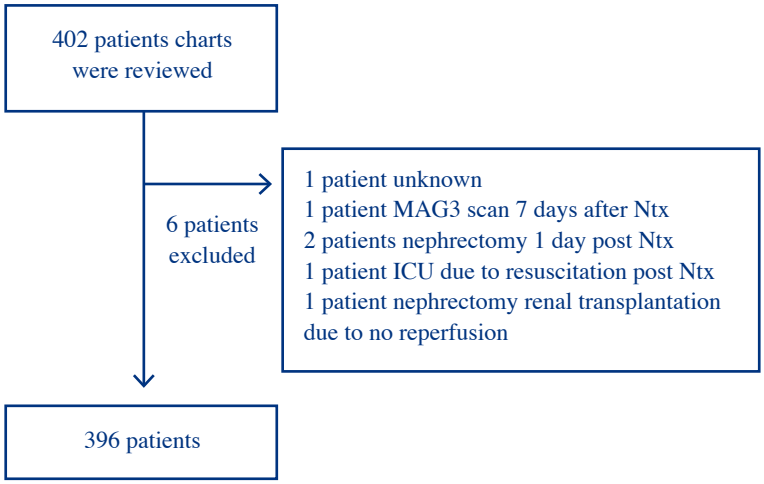

Table 1 - Baseline characteristics of the patients. *

Characteristics	Value (n=396)
Patients age – yr. **	56 (17-79)
Male sex – no. (%)	247 (62.4)
Original kidney disease – no. (%)	
Glomerulonephritis	62 (15.7)
Diabetes mellitus	69 (17.4)
Urological disorder	17 (4.3)
Hypertension/vascular damage	79 (19.9)
Polycystic kidney disease	49 (12.4)
Uncertain	52 (13.1)
Other reason	68 (17.2)
Type of donor – no. (%)	
Living	266 (67.2)
Postmortal – donation after circulatory death	73 (18.4)
Postmortal – donation after brain death	57 (14.4)
1st renal transplantation – no. (%)	324 (81.8)
Donor age – yr **	55 (0-89)
HLA-antigen mismatches – A, B and Dr (no.)	3.6 ± 0.08

*Values are presented as means ± standard deviation.
**Values are presented as mean (range).

Patients with urinary leakage
There were 3 patients with a urinary leakage on the protocol MAG3 scan. 1 patient was diagnosed based on clinical appearance, abdominal pain and more than 1 liter urine in the drain. A PCN drain was placed in 1 patient due to the result of the MAG3 scan. After the placement of the PCN drain the patient got abdominal pain and cold shivers. Finally, the results of the protocol MAG3 scan led to the performance of a new MAG3 scan in 1 patient, which showed no urinary leakage. To confirm that the clinical decision was based on the MAG3 scan, we also evaluated the LD (lactate dyhydrogenase)-levels. 2 patients had a normal LD-level. 1 patient, the patient who underwent a second MAG3 scan, had an elevated LD-level 3 and 8 days after transplantation.

Table 2 - Baseline characteristics of the patients, according to the result of the MAG3 scan.*

Variable	Result of the protocol MAG3 scan		p-Value
	Normal (n=219)	Abnormal (n=177)	
Patients age – yr.**	55 (18-77)	57 (17-79)	0.053
Male sex – no. (%)	131(59.8)	116 (65.5)	0.24
Original kidney disease– no. (%)			0.06
Glomerulonephritis	39 (17.8)	23 (13.0)	
Diabetes mellitus	28 (12.8)	41 (23.2)	
Urological disorder	9 (4.1)	8 (4.5)	
Hypertension/vascular damage	46 (21.0)	33 (18.6)	
Polycystic kidney disease	32 (14.6)	17 (9.6)	
Uncertain	24 (11.0)	28 (15.8)	
Other reason	41 (18.7)	27 (15.3)	
Type of donor – no. (%)			<0.01
Living	193 (88.1)	73 (40.9)	
Postmortal – donation after circulatory death	11 (5.0)	62 (35.0)	
Postmortal – donation after brain death	15 (6.8)	42 (23.7)	
1st renal transplantation – no. (%)	183 (83.6)	141 (79.7)	0.34
Donor age - yr **	51.5 (14-89)	58 (0-86)	<0.01
HLA-antigen mismatches – A, B and DR – no.	3.50 ± 1.75	3.63 ± 1.54	0.98
Multiple arteries donor – no. (%)	48 (21.9)	43 (24.3)	0.58
Vascular reconstruction	47 (21.5)	43 (24.3)	0.50
Extrarenal pyelum donor – no. (%)	8 (3.7)	3 (1.7)	0.24
Cold ischemia time (only postmortal donors) – hr.	14.0 ± 4.8	13.7 ± 4.7	0.68
Warm ischemia time – min. **	19 (10-58)	22 (8-52)	<0.01
Influence on clinical outcome – no. (%)	0 (0)	31 (17.5)	<0.01

*Values are presented as means ± standard deviation.
**Values are presented as mean (range).

Table 3 - Influence on clinical decision making. *

Variable	Influence on clinical decision making		p-Value
	Yes (n=31)	No (n=365)	
Patients age – yr. **	56 (25-75)	56 (17-79)	0.65
Donor age – yr. **	58 (24-72)	55 (0-89)	0.21
Male sex – no. (%)	20 (64.5)	227 (62.2)	0.80
Original kidney disease – no. (%)			0.75
Glomerulonephritis	4 (12.9)	58 (15.9)	
Diabetes mellitus	3 (9.7)	66 (18.1)	
Urological disorder	2 (6.5)	15 (4.1)	
Hypertension/vascular damage	9 (29.0)	70 (19.2)	
Polycystic kidney disease	4 (12.9)	45 (12.3)	
Uncertain	3 (9.7)	49 (13.4)	
Other reason	6 (19.4)	62 (17.0)	
Type of donor– no. (%)			0.054
Living	15 (48.4)	251 (68.8)	
Postmortal – donation after circulatory death	10 (32.3)	63 (17.3)	
Postmortal – donation after brain death	6 (19.4)	51 (14.0)	
1st renal transplantation – no. (%)	26 (83.9)	298 (81.6)	0.03
Antigen mismatches – A, B and DR – no.	3.68 ± 1.45	3.55 ± 1.68	0.79
Multiple arteries donor – no. (%)	11 (35.5)	80 (21.9)	0.09
Vascular reconstruction	11 (35.5)	79 (21.6)	0.08
Extrarenal pyelum donor – no. (%)	0 (0)	11 (3.0)	0.33
Cold ischemia time (only post-mortem donors)- hr.	13.13 ± 3.64	13.82 ± 4.83	0.46
Warm ischemia time – min.**	21 (13-50)	20 (8-58)	0.026

*Values are presented as means ± standard deviation
**Values are presented as mean (range).

All 3 patients had a decreased creatinine level 3 months after transplantation compared to their creatinine level 1day post transplantation. Nevertheless none of these creatinine levels were in the normal range (men 45 – 100 µmol/L, women 45 – 80 µmol/L) (table 4).

Patients with a kidney infarct

5 patients were suspected of a kidney infarct based on the protocol MAG3 scan. 1 patient had a hematoma 1day post transplantation. Also, during the re-exploration surgery a perfusion defect was seen. This defect was seen on the protocol MAG3 scan as well. 2 of the patients with a possible kidney infarct on the protocol MAG3 scan were excluded from having a kidney infarct with a SPECT-CT. 1 patient was excluded from having a kidney infarct by performing an ultrasound and 1 patient did not receive further diagnostic imaging to confirm the small defect seen on the protocol MAG3 scan. Evaluation of the creatinine levels after 3 months showed that all 5 patients had a decreased creatinine level compared to creatinine level post transplantation. Nevertheless, none of the creatinine levels were in the normal range (table 4).

Discussion

Our data show that of all 396 patients who underwent renal transplantation, 177 (44.7%) had an abnormal MAG3 scan. However, in only 31 (7.8%) patients the MAG3 scan had an influence on patient care. We noted 3 differences between patients with a normal MAG3 scan and patients with an abnormal MAG3 scan. A significant difference was observed in the age of the donor, the donor type and warm ischemia time. All 3 characteristics influence the amount of ischemia reperfusion injury and hence could lead to tubula necrosis and delayed graft function, which will result in its turn in an abnormal MAG3 scan [8-11]. Indeed, a decrease in extraction or excretion, which represents tubular ischemia, was the most commonly reported abnormality (n=139). The lack of influence on the renal anatomy or the need for vascular reconstruction on the results of the MAG3 scan was striking. Performing a vascular reconstruction (n=90 in our analysis) is known to increase the risk of perfusion defects [12]. Moreover, a recent study from Sagban et al, including 1134 patients, concluded that multiple renal arteries are associated with a longer warm ischemia time, decrease in graft function and acute tubular necrosis [13]. Although small perfusion defects can be missed on a MAG3 scan, it is likely that thrombosis of a reconstructed vessel would result in large perfusion defect, which would probably not go undetected. A lack of effect on (regional) kidney perfusion (and hence on the results of the MAG3 scan) might therefore be explained by the routine use of heparin during 3 days, which is common practice in The Erasmus Medical Center Rotterdam. Moreover, although vascular reconstruction could lead to a compromised blood supply to the urether and the urether-bladder anastomosis resulting in either fibrosis or leakage, this would probably not be detectable during the first day post-transplant.

Table 4 - Patients with a urinary leakage or kidney infarction

Diagnosis	Based on	Creat post transplantation	Creat after 3 months
1 Urinary leakage	Clinical indication; abdominal pain and >1 L urine in drain. Decided to re-operate, no urinary leakage was found. 1 day post re-operation MAG3 scan showed urinary leakage.	221	107
2 Urinary leakage	On protocol MAG3 scan urinary leakage was seen, there was no clinical indication, although urinary production was not immediately present.	1765	278
3 Urinary leakage	On protocol MAG3 scan urinary leakage was seen, there was no clinical indication.	262	155
4 Kidney infarct	On protocol MAG3 scan small defect was seen, possibly a kidney infarct.	307	101
5 Kidney infarct	On protocol MAG3 scan possible cortex defect was seen.	867	101
6 Kidney infarct	On protocol MAG3 scan a photon poor area was seen. Possible a kidney infarct.	477	160
7 Kidney infarct	On protocol MAG3 scan area with less activity was seen. Limited infarction not excluded.	257	121
8 Kidney infarct	On protocol MAG3 scan area with less activity was seen. Limited infarction not excluded. 1 day post-transplantation patient underwent a re-exploration because of a hematoma. During this operation a closed artery was seen with a perfusion defect.	856	138

Since a MAG3 scan is not only costly (approximately €400) but also a burden for patient and nurses, limiting the protocol MAG3 scan to high-risk patients would be more (cost-) efficient. Despite clear differences in the 3 abovementioned characteristics, no cut-off point has been found. For example, although the percentage of postmortal donors was significantly higher among patients with an abnormal MAG3 scan, exclusion of recipients of a living donor kidney, would have meant that clinically relevant outcomes would have been missed in 15 patients.

A strength of this study is that all patients who underwent a kidney transplantation between January 2013 and January 2015 are included, which limits bias. Also, the influence of the result of the MAG3 scan on the clinical outcome has been viewed over 4 weeks, so there is a small chance that a result is missed. However, there is a possibility that changes in clinical outcome are not always noted in the medical charts. This would have led to an underestimation of the influence on clinical care. In some patients the influence on patient care was more profound than in others. Other diagnostic testing could have influenced clinical decision making. For example, since a standard ultrasound was also performed, which also has the ability to detect perfusion defects, the sole contribution of the MAG3 scan in the decisions around the renal transplant patient care was not always clear. The only way to properly address all concerns in this trial is to perform a controlled trial, and to randomize for performing or withholding a protocol MAG3 scan.

In conclusion, in 31 of 396 patients, a protocol MAG3 scan led to a change in renal transplant patient care. In making the diagnosis of a renal infarction or urinary leakage, the MAG3 scan only aided in 3 patients. Although patients with an abnormal MAG3 scan received a kidney from a donor with significantly higher age, more frequently from a postmortal donor and with a longer warm ischemia time, these characteristics could not sufficiently predict the outcome of the protocol MAG3 scan. Therefore, these scans could not be used as a selection criterion to limit the patient population in which to perform a protocol MAG3 scan. Since a MAG3 scan is not only costly but also a burden for patient and nurses, our data suggest that the role of a protocol MAG3 scan in renal transplant patient care is very limited.

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Systematic Review

Mesenchymal stem cells for the modulation of implant site in intra bone marrow pancreatic islet transplantation

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Abstract

Background: **Type 1 Diabetes (T1D) is an autoimmune disease caused by a progressive auto reactive immune response against islets resulting in a permanent destruction of β-cells and thereby the inability to produce insulin1. Mesenchymal stem cells (MSC) are known for the potential to modulate immune responses and to enhance engraftment if co-transplanted with islets. The bone marrow (BM) has been recently proposed as an alternative site for islet transplantation. The BM, due to its structure and anatomical position, offers the possibility to modulate microenvironment by local interventions.**

Methods: **In the current study, three groups of alloxan induced severely diabetic C57BL/6 mice were transplanted with islets from BALB/c mice and co-transplanted with syngeneic BM derived MSC, or pancreatic derived MSC (pMSC) to investigate whether MSC co-transplanted in mice are able to improve islet engraftment and prevent rejection in the BM. The BM was irradiated to create a niche for engraftment. The primary readout parameters during the 1 month experimental period were blood glucose level; islet engraftment (defined as achievement of not fasting glycaemia <300 mg/dl) and graft rejection, (defined as two consecutive measurements >350 mg/dl).**

Results: **The results showed a significant improved effect on engraftment in the first three days when BM-MSC are co-transplanted with allogeneic islets compared to pMSC and islets or islets alone. No significant effect on allograft rejection was observed at any time point during the experiment. The BM-MSC tracing in vivo through the Carboxyfluorescein succinimidyl ester (CFSE) staining demonstrated that no BM-MSC engraft in the BM.**

Conclusion: **In conclusion, this study demonstrates an improved engraftment when islets are co-transplanted with BM-MSC into the locally irradiated BM compared to pMSC and islets alone CFSE staining showed that no BM-MSC engraft in the BM and thus suggests that this favourable effect is most likely due to the impact of transitory paracrine effects rather than local effects.**

Discussion: Although we found improved engraftment we were not able to postpone allograft rejection. This might be due to the stringent C57BL/6 mouse model, the migration of BM-MSC or other factors which need to be identified.

***Keywords*: BM-MSC, pMSC, islet transplantation, BM, T1D**

Introduction

Type 1 diabetes (T1D) is an autoimmune disease caused by a progressive auto reactive immune response against islets resulting in a permanent destruction of β-cells and thereby the inability to produce insulin[1]. Although insulin therapy has improved patients' quality of life, severe hypoglycaemic episodes may occur and chronic diabetic complications, such as nephropathy, retinopathy and cardiovascular diseases are extremely frequent[2,3]. Islet transplantation represents an important therapeutic option for adult patients with unstable T1D with an increased risk for acute and chronic complications.

The first islet transplantation was performed in 1973 in the intra-hepatic site, through the portal vein4. In the last years, it has been recognized that the intra-hepatic site may not provide the ideal microenvironment for islets due to anatomic[5-8], physiologic[5,8,9], and immunologic factors[10], for instance an instant blood mediated inflammatory reaction (IBMIR)[11]. IBMIR is a thrombotic innate reaction which causes clot formation and leukocyte infiltration into the islets and hepatic ischemia with elevated blood liver enzymes[12]. It is estimated that 50-60% of the graft is lost early after transplantation mainly due to this inflammatory reaction[13,14]. An additional important limitation is the increase in portal vein pressure by the infusion of islets and thus restricting the mass that can be transplanted[7]. To overcome this problem a high purity of islets is required and thus more donors are needed from the already scarce donor pool[6,7]. Altogether the recognition of these factors has contributed to an increased interest in the search for an alternative site.

Bone marrow (BM) has recently been proposed as an alternative site for islet transplantation[15]. The BM consists of hematopoietic, mesenchymal (stromal) and endothelial cell precursors, which can contribute to tissue repair/remodeling, engraftment and suppression of inflammatory responses[16]. The BM is an extravascular site and thus prevents IBMIR. Besides, it is widely distributed and easily accessible for the surgical procedure, thus overcoming the technical limitations/complications of intra-hepatic infusion[17]. A preclinical study showed increased rates of success in reversing hyperglycaemia after syngeneic-islet transplantation into the BM compared to intra-hepatic infusion[17]. Additionally, Maffi et al. performed for the first time auto-islet infusion in a clinical setting and confirmed as well successful engraftment and functioning with no adverse events after islet infusion in the BM[18].

An effective strategy to prevent rejection and improve engraftment is the co-transplantation of MSC with the islets. For example, in a islet allograft mouse model, MSC co-transplanted and co-localized under the kidney capsule protected islets grafts leading to long-term islet allograft function[19,20] by blocking the activation and the expansion of alloreactive T cells through secreting the immunosuppressive matrix metalloproteinase-2 and -9[19]. Co-localization of MSC and target tissue in the same microenvironment is most important. MSC are the best candidate for cell therapy supporting islet transplantation. In fact in the last years it became apparent that in many situations MSC induce repair and functional improvement in injured tissues[21]. This happens without significant engraftment or differentiation, but is probably due to a role of feeder cells. MSC in culture secrete a large number of cytokines, chemokines and other factors[22]. In particular, MSC release several factors that support β-cell survival and function: they were described to secrete laminins binding to alpha 6 beta 1-integrin expressed on pancreatic β-cells which support insulin expression and β-cell proliferation[23], they secrete collagen IV binding to alpha1 beta 1-integrin which stimulates insulin secretion[24], they release trophic molecules like hepatocyte growth factor (HGF), interleukin-6 (IL-6), insulin-like growth factor binding protein 4, vascular endothelial growth factor A (VEGF A) and transforming growth factor-beta (TGF-β) that can directly sustain β-cell survival and function and induce neovascularization[25].

Another important aspect in favor of the use of MSC in islet transplantation is their well-established suppressive capabilities that may be exploited to control several subsets of immune cells, including naïve and memory T cells[26], B cells[26], dendritic cells[27], NK cells and to reduce inflammatory cytokine production[28,29]. In islet transplantation, MSC could protect transplanted allogeneic islets by negatively regulating persistent T cell autoimmunity and by controlling the activation and effector function of alloreactive T cells[26]. In addition, MSC may also suppress the activation and proliferation of B cells thereby impairing the production of destructive auto and allo-antibodies and may prevent differentiation and maturation of DC, thus preventing destruction of transplanted islets[26].

Our lab identified a new subset of MSC, the pancreatic mesenchymal stem cells (pMSC)[20]. Considering the large number of pMSCs obtainable from digested pancreas, they could be useful as islet "helper" cells and might have thereby a beneficial effect on the micro-environment in the BM. As far as we know the effects of co-transplantation with pMSC compared to BM-MSC into the BM have not been studied. The current study has been set up to investigate in a preclinical setting, the effect on islet engraftment and allograft rejection when pMSC are co-transplanted or when BM-MSC are co-transplanted.

Material and methods

MSC culture, characterization and tracing

Bone marrow mesenchymal stem cell (BM-MSC)

Commercially BM-MSC from C57BL7/6 mice were used from GIBCO (Invitrogen, Carlsbad, USA). Cells were cultured in Eagle's minimum essential medium, alpha modification (α-MEM) supplemented with 1% L-glutamine (Sigma-Aldrich, Saint Louis, USA), 1% penicillin-streptomycin (Sigma-Aldrich) and 10% fetal bovine serum (Euroclone, Milano, IT). The cells were plated in T75 tissue culture treated flask (Costar, Glasgow, UK) and grown at 37°C in a humidified incubator at 5% CO2. Medium was changed every 3 days. Cells were harvested by trypsinization.

Pancreatic mesenchymal stem cell (pMSC)

Primary pancreatic tissues were obtained from the digest remaining after pancreatic islet isolation from pancreata of 8-week-old C57BL7/6 male mice. Following two washes in phosphate buffered solution (PBS), the equivalent of 0.1 ml of packed pellet was resuspended in α-MEM supplemented with 1% L-glutamine (Sigma-Aldrich), 1% penicillin-streptomycin (Sigma-Aldrich) and 10% fetal bovine serum (Euroclone). Medium was changed every 3-days. Cells were harvested by trypsinization and replated at a density of 2,500 cells/cm2 for up to 30 passages (200 days). pMSC were characterized for their capacity to differentiate to adipocytes and osteoblasts with a hMSC Differentiation BulletKit (Cambrex, Milano, IT) according to the manufacturer's instructions. BM-MSC and pMSC were photographed under an inverted microscope (Leica DMIRB equipped with a Leica DC300Fx digital camera, Wetzlar, DE).

Characterization of MSC

Cells were stained with a live/dead fixable dead cell stain kit (Invitrogen) and then directly stained using fluorochrome-conjugated monoclonal antibodies (mAbs). The following mAbs were used: FITC rat anti-mouse Sca1 (clone D7), PE rat anti-mouse CD90.2 (clone 30-H12), FITC rat anti-mouse CD31 (clone MEC13.3, RUE), APC rat anti-mouse c-kit (clone 2B8), FITC or PerCP-Cy5-5 rat anti-mouse CD45 (clone 3-F11), APC rat anti-mouse CD44 (clone IM7) (BD Pharmingen, San Jose, USA). Flow cytometry was carried out on a BD FacsCanto II flow cytometer using FACSDiva software (BD Biosciences, San Jose, USA).

Irradiation, islet isolation, transplantation and graft evaluation
Mice
Male C57BL/6 mice (8 weeks old, Charles River Laboratories, Tranent, UK) were used as recipients. Diabetes was induced (not fasting glycaemia >450mg/dL) with intravenous alloxan injection (75g/kg, Sigma-Aldrich) 6 days before transplantation. Blood glucose measurements were performed using a Glucometer Elite (Bayer, Barmen, DE). The animals had free access to tap water and pelleted food throughout the course of the study. The animal ethics committee of San Raffaele Scientific Institute approved all experiments.

Islet isolation
Pancreatic islets were isolated from BALB/c mice (8 weeks old, Charles River Laboratories) by a collagenase digestion method. Briefly, 2 mL of cold Hanks buffer/collagenase type V solution (1 mg/mL, Sigma-Aldrich) was infused into the pancreatic duct in situ and the removed pancreas was digested at 37°C for 15 minutes. Islets were purified on a discontinuous Ficoll gradient (Sigma-Aldrich). The islets (250 islets/mL) were cultured freely floating (37°C, 5% CO2) in medium RPMI 1640 (Euroclone) supplemented with 1% L-glutamine (Sigma-Aldrich), 1% penicillin-streptomycin (Sigma-Aldrich), and 10% fetal bovine serum (Euroclone) for 20 to 24 hours before the transplantation. Islet purity was more than 90%.

Local irradiation of the BM
A selective irradiation model was set up in order to create space in the BM cavity to improve islet and MSC engraftment. Another purpose of this experiment was to verify whether the irradiation was local and selective.The right femur of severely diabetic C57BL/6 mice was selectively irradiated by a partial body fixture and shield. Irradiated C57BL/6 mice were exposed to 0,8 Gy/min for 12 minutes, and the procedure was repeated after 2 hours.

Intra-BM transplantation of islets alone and islets + pMSC or BM-MSC
Islets were transplanted in the BM as follows: recipients were anesthetized with avertin. A 0.5-cm longitudinal incision was made in front of the right knee and the plate of the femur was exposed and trepanized with a 3/32-inch shank carbide burr in the direction of the medullar channel. 450-500 Islet Equivalent (IEq) alone or together with 200.000-250.000 pMSC/ BM-MSC packed in PE-50 polyethylene tubing (BD Biosciences) were then introduced into the medullar channel. When the tubing was withdrawn, the cluster of islets and MSC was left in the medullar channel by the vacuum effect. The skin was closed with 4-0[17].

Evaluation of graft function
Blood glucose levels were measured 0, 15, 30 and 60 minutes after the end of the surgical procedure. Thereafter daily for the first two weeks and from then on every second day, until 1 month after transplantation. Islet engraftment was defined as achievement of not fasting glycaemia <300 mg/dl and graft rejection was defined as two consecutive measurements >350 mg/dl in mice that have achieved normoglycaemia.

MSC labeling with CFSE and in vivo tracing
In order to trace in vivo BM-MSC, the cells were stained with 0.5uM Carboxyfluorescein succinimidyl ester (CFSE, Molecular Probes, Paisley, UK) according to the manufacturer's instructions. For the set-up of the experiment two preliminary experiments were performed: 1) In order to define the rate of detection of labelled BM-MSC mixed with BM cells, we isolated BM cells from a C57BL/6 mouse and diluted these with CFSE-labelled BM-MSC (starting with 1.000.000 cells). Dilution was performed by a factor 2, until a dillusion-factor of 64 was reached. 2) To verify whether it was possible to track labelled MSC after infusion into the BM, we transplanted 250.000 BM-MSC labelled with CFSE in the BM of a C57BL/6 mouse. We sacrificed the mouse immediately after infusion. We isolated BM cells from the transplant site and the contralateral BM by femur flush and analysed them by flow cytometry. For the final experiment CFSE labelled MSC were transplanted in the irradiated BM alone or together with the islets. Recipient mice were sacrificed immediately after transplantation-, 4-, 24- and 72 hours. Spleen, BM-, (both the transplant and the contralateral site) and blood were analyzed. Splenocytes were obtained by cutting the capsule of the spleen and the spleen was mashed with the plunger end of a syringe while adding PBS. Cell suspension was filtered with a 70μm filter and red blood cells were lysed using ACK lysis buffer. The cell suspension was filtered with a 40μm filter. Blood was obtained from the retro-orbital plexus

Flow cytometry
Splenocytes, BM and blood cells were stained with a live/dead fixable dead cell stain kit (Invitrogen). Flow cytometry was carried out on a BD FacsCanto II flow cytometer using FACSDiva software (BD Biosciences).

Statistical analysis
Data were expressed as mean ± standard error. Variables with a normal distribution were compared with Student's t-test. Variables with a non-normal distribution were compared with Mann Whitney U-test. Pearson $\chi 2$ test was used to compare proportions. The time to graft rejection was evaluated by the Kaplan-Meier analysis, and the significance was estimated by the log-rank test. Data were analyzed using the SPSS statistical software, version 13.0 (SPSS Inc., Chicago, IL, USA).

Results

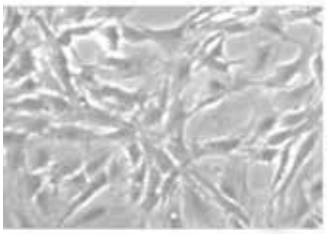
1. Flowcytometry characterization of MSC
1.1 BM-MSC
BM-MSC were analyzed for a combination of surface markers that define MSC. The BM-MSC were positive for CD44 (97,4%), CD90.2 (29,7%), sca-1 (96,8%), negative for CD45 (6,0%), c-kit (4,4%), and slightly positive for CD31 (15,7%) (Fig 1.A).

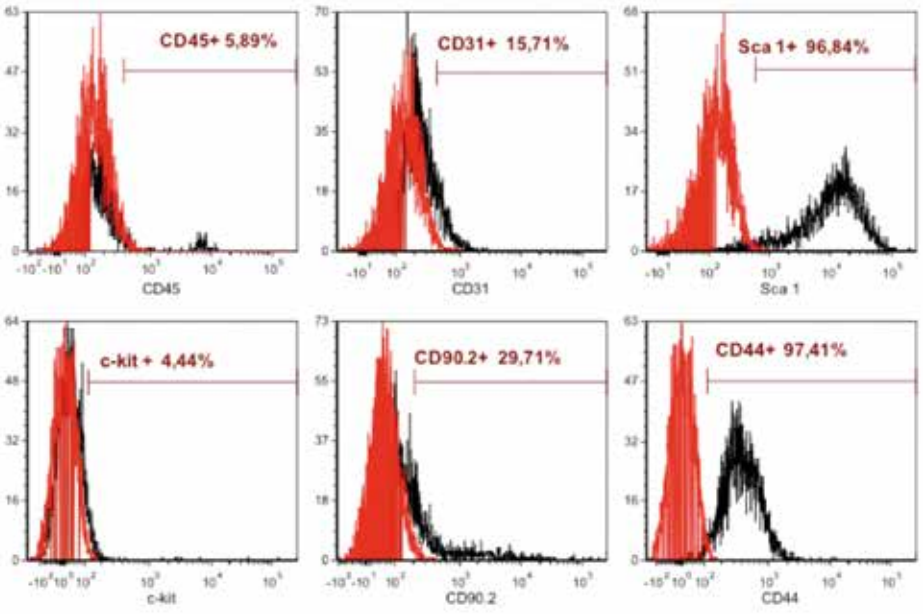
1.2 pMSC
pMSC were positive for CD44 (99,3%), CD90.2 (93,5%), sca-1 (90,7%) and negative for CD45 (5,7%) and slightly positive for c-kit (19,2%), CD31 (14,0%) (Fig 1.B).

Figure 1a- Characterization of mouse BM-derived and pancreatic derived MSC

BM-MSC C57BL/6

BM derived MSC

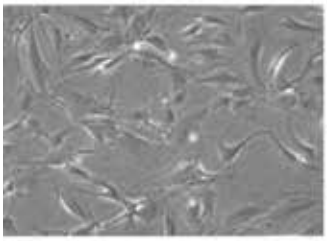




Morphology (magnification 10x, 3.10 pixels/um) and expression profile of surface antigens of MSC. Specific surface markers (black), unstained sample (red).

Figure 1b

pMSC



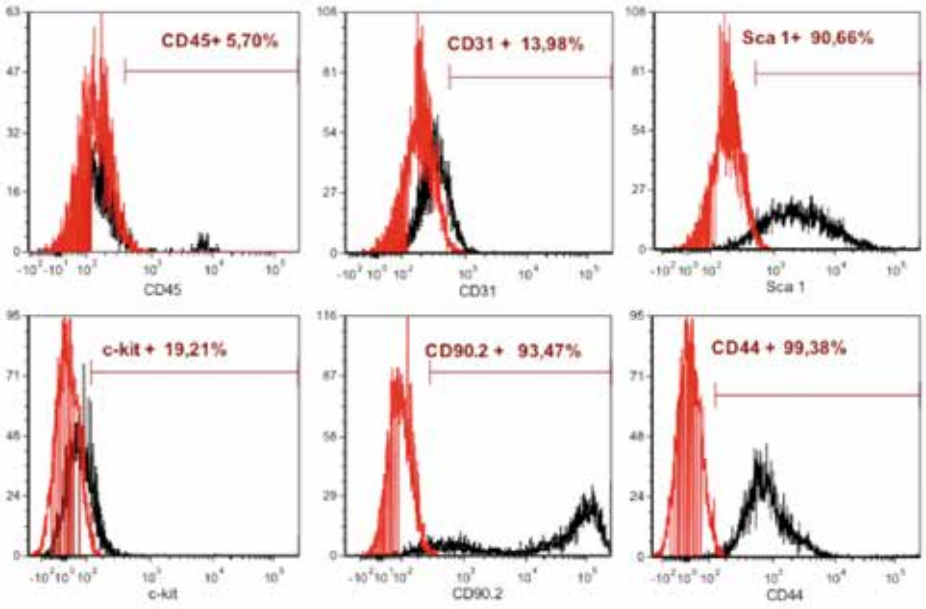
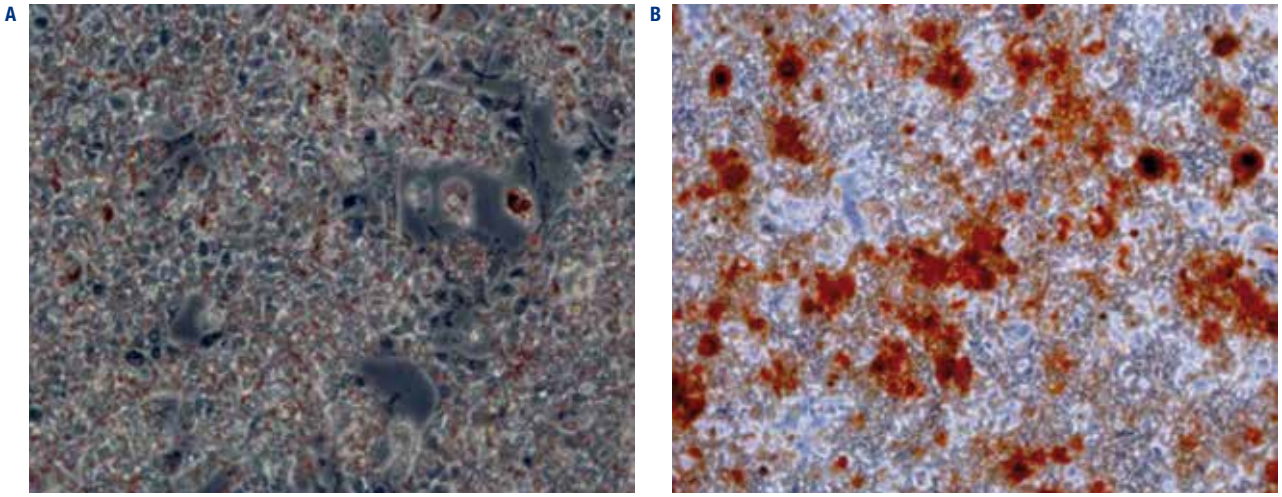


Figure 2 - Differential potential of pMSC

A: Adipogenic (alizarin staining) B: Osteogenic (oil red staining)



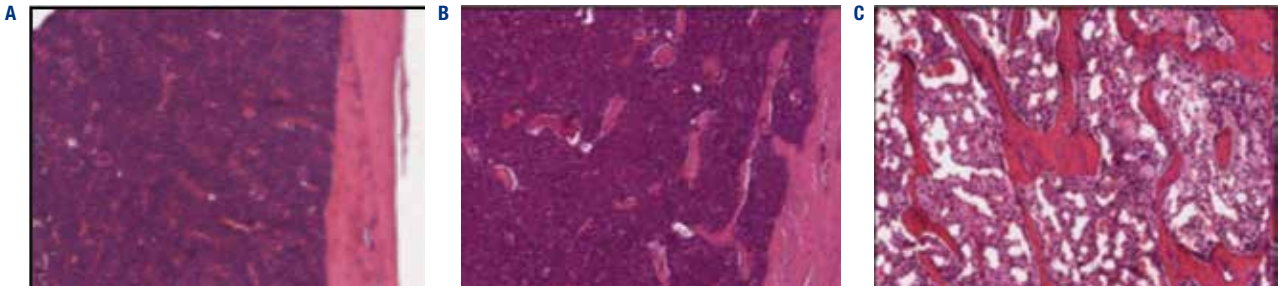
PMSC were differentiated into adipocytes and osteocytes (magnification 20x, 1.55 pixel/um).

To confirm that pancreatic-derived cells were MSC, the cells were differentiated into adipocytes (Fig 2.A) and osteocytes (Fig 2.B) under standard differentiation conditions. Phenotypically pMSC expressed the same markers as the BM-MSC, except for CD90.2. In fact CD90.2 can be expressed differently in different subsets of MSC30. This is in accordance with previous pMSC characterizations in our lab[20].

2. Histological characterization of locally irradiated BM

From five alloxan-induced diabetic C57BL/6 mice two were non-irradiated (n=2) and three were locally irradiated (n=3). Both locally irradiated BM (Fig 3.C) and non-irradiated BM (contralateral site) (Fig 3B.) were analyzed by histology 3 days after the irradiation. Non-irradiated BM from diabetic mice were used as control (Fig 3A.). Histology identified a selective cell depletion of the locally irradiated BM compared to the non-irradiated contralateral BM and the BM of the control mice.

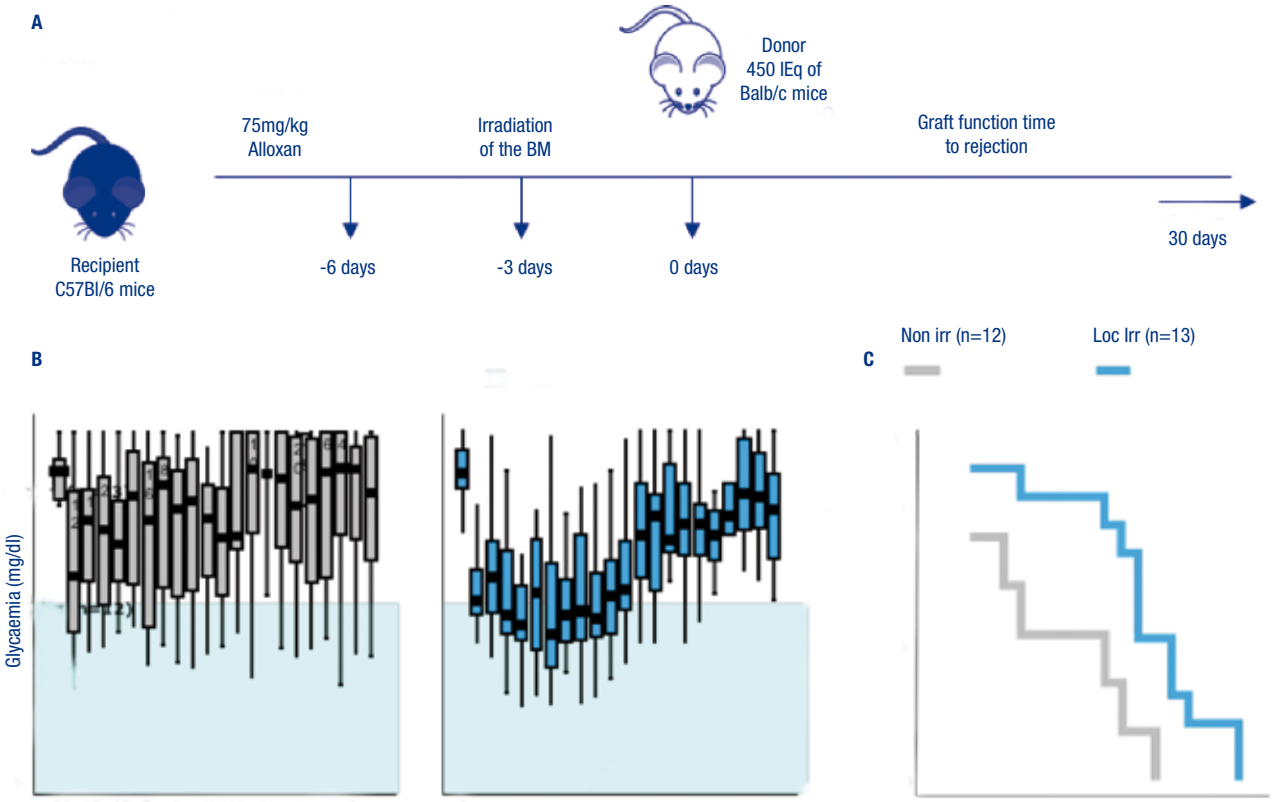
Figure 3 - Histological effect of locally irradiated BM



Haematoxylin & eosin staining. Performed by Cantarelli E, Sordi V and Pellegrini S.

A: BM of non-irradiated diabetic C57BL/6 mouse (magnification 10x, 3.10 pixels/um)
B: BM of contralateral site from the locally irradiated diabetic C57BL/6 (magnification 20x, 1.55 pixel/um)
C: BM of locally irradiated diabetic C57BL/6 mouse (magnification 20x, 1.55 pixel/um)

Figure 4 - Allogeneic islet transplantation in locally irradiated BM

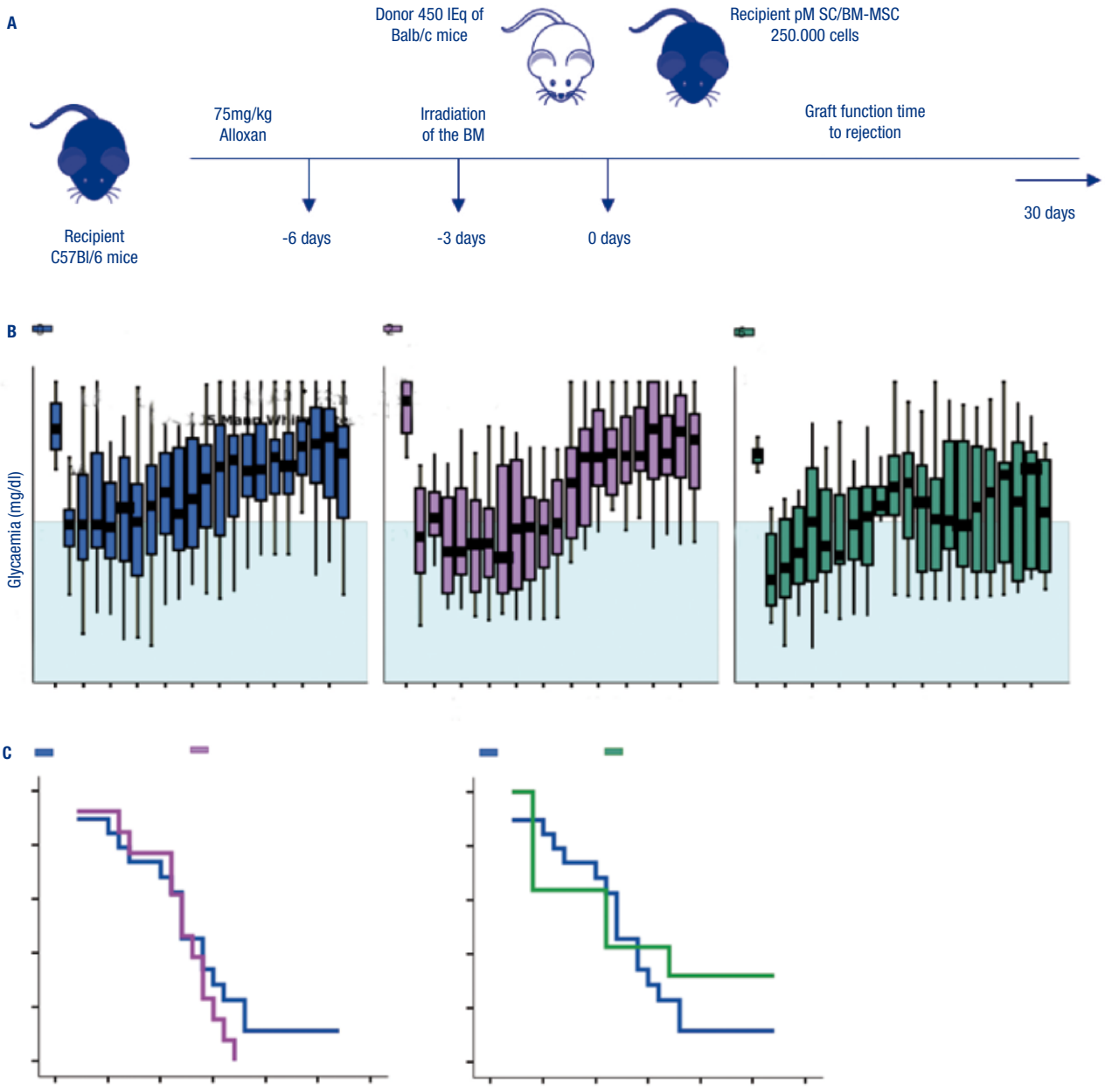


A: Experiment planning.
B: Box plots of not fasting glycaemia levels (mean) in the days after transplantation.
Non-irradiated (non irr) mice (gray); locally irradiated (loc irr) mice (blue). Statistical analysis was performed by tests of repeated measures ANOVA. The blue window represents normoglycaemia achievement after transplantation.
C: Kaplan-Meier analysis for graft rejection. Differences between non irr and loc irr groups were tested using a log rank statistic test.

3. Allogeneic islet transplantation in locally irradiated BM

Twenty five alloxan-induced diabetic C57BL/6 mice (locally irradiated, n=13) or (non-irradiated, n=12) were transplanted with 450 Balb/c IEq (Fig 4.A). Twelve out of 13 (92%) and 7 out of 12 (58%) mice achieved normoglycaemia after islet transplantation (p=0,069). Islet engraftment was improved during the first eleven days after transplant for mice with local irradiation of the BM (p<0,05) compared to non-irradiation of the BM (Fig 4.B.). In the recipients that achieved normoglycaemia after islet transplantation, time to graft rejection was monitored. The median time to graft rejection was 12 ± 0.6 days for locally irradiated mice and 5 ± 3.3 days for non-irradiated mice (p<0,01, Fig 4.C).

Figure 5 - Allogeneic transplantation of islets alone, islets + pMSC and islets + BM-MSC in loc irr BM.



A: Experimental plan.
B: Box plots of not fasting glycaemia levels (mean) in the days after transplantation. Loc irr mice transplanted with islets alone (blue); loc irr mice transplanted with islets + pMSC (purple); loc irr mice transplanted with islets + BM-MSC (green). Statistical analysis was performed by tests of repeated measures ANOVA. The blue window represented normoglycaemia achievement after transplantation.
C: Kaplan-Meier analysis for graft rejection. Differences between groups were tested using a log rank statistic test

4. Co-transplantation of autologous BM-MSC or pMSC with allogeneic islets into locally irradiated BM vs islets alone

Fifty-one alloxan-induced diabetic and locally irradiated C57BL/6 mice were co-transplanted with 450 Balb/c IEq alone (n=23) and in combination with 200.000-250.000 pMSC (n=15) or BM-MSC (n=13).

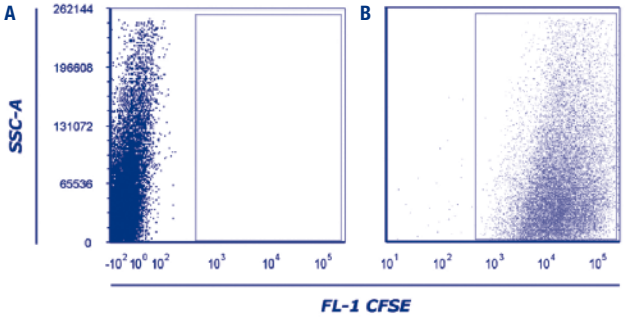
The numbers of mice that gained normoglycaemia were: 11/15 (73%), 8/13 (62%) and 16/23 (70%) respectively for islets + pMSC, islets + BM-MSC and islets alone. Islet engraftment was improved during the first three days after transplant for mice co-transplanted with islets and BM-MSC ($p<0,05$) compared to islets alone and at day 2 after transplant for mice co-transplanted with islets and pMSC ($p<0,05$) compared to islets alone (Fig 5.B.). The median time to graft rejection was: 12 ± 1.24 days for mice transplanted with islets alone, 11 ± 5.15 days for mice transplanted with islets + BM-MSC and 12 ± 1.20 days for mice transplanted with islets + pMSC (Fig 5.C). Despite a beneficial effect on islet engraftment in the first days after transplant, the time to graft rejection was not delayed when islets were co-transplanted with BM-MSC or pMSC as compared to islets alone.

5. Tracking BM-MSC labelled with CFSE

5.1 Set-up of the experiment

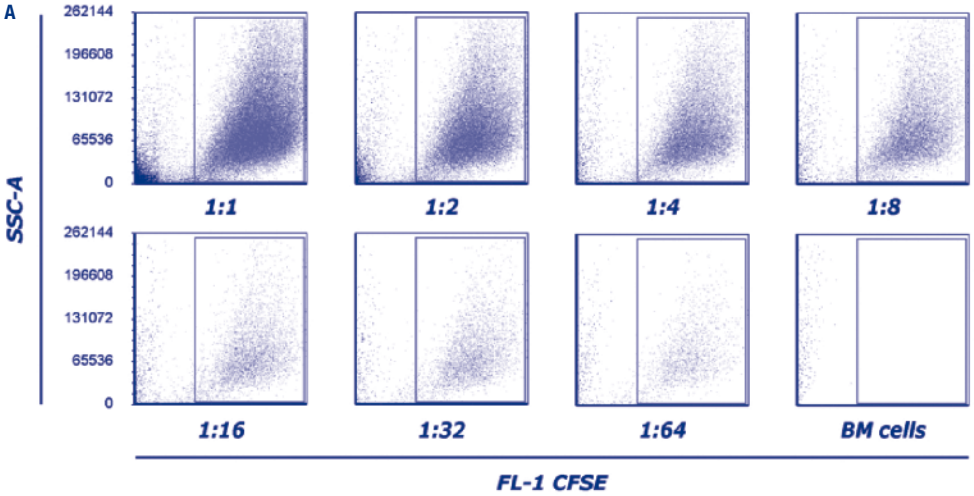
1) To define the rate of detection of labelled BM-MSC mixed with BM cells, we isolated BM cells from a C57BL/6 mouse and diluted with CFSE-labelled BM-MSC (starting with 1.000.000 cells). Dilution was performed by a factor 2, until a dillusion-factor of 64 was reached. We detected 20% CFSE+ BM-MSC in the 1:2 dilution and <0.3% CFSE+ BM-MSC in the 1:64 dilution. The detection of CFSE+ BM-MSC more or less halved by each dilution step. This experiment verifies that labelled MSC can be detected even at higher dilutions (Fig 6 and 7.A); 2).

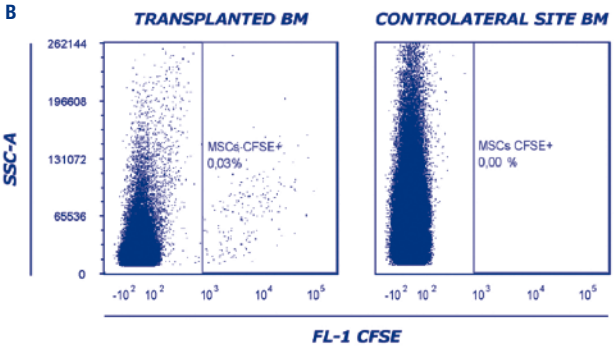
Figure 6 - Set-up for the flow cytometry analyses to identify MSC labelled with CFSE.



BM-MSC were stained with CFSE for cell tracing experiments.
A: dot plot shows unstained B: dot plot shows CFSE labelled cells

Figure 7 - Identification of BM-MSC labelled with CFSE.

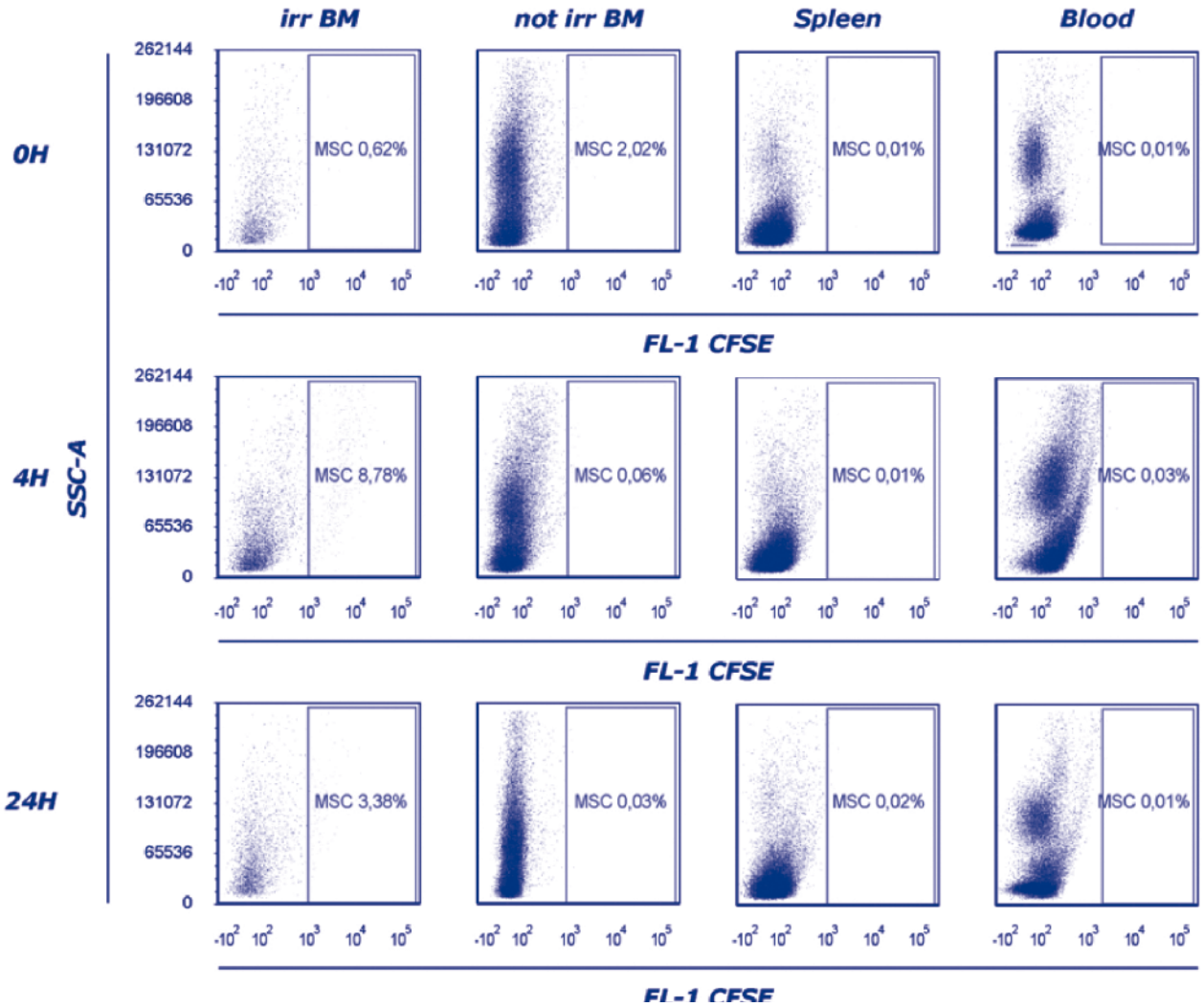




2) To verify whether it was possible to track labelled MSC after infusion into the BM, we transplanted 250.000 BM-MSC labelled with CFSE in the BM of a C57BL/6 mouse. We sacrificed the mouse immediately after infusion. We isolated BM cells from the transplant site and the contralateral. In the contralateral BM, CFSE+ MSC were not detected. In the transplanted BM, we detected 0,03% CFSE+ MSC. Thus, BM-MSC can be traced in vivo by using CFSE labelling and can be detected immediately after transplantation in the BM of healthy mice (Fig 7.B).

A: Detection of MSC in different dilution steps with BM-cells in vitro.
B: Detection of CFSE+ MSC after MSC infusion in the transplanted BM and the not transplanted (contralateral site)

Figure 8 - In vivo tracking of CFSE-labelled BM-MSC transplanted in locally irradiated BM



Analysis of C57BL/6 mice transplanted with syngeneic MSC CFSE labelled cells 0-, 4-, and 24-hours after transplantation.
MSC CFSE+ are presented as a percentage of live cells (1 of the 2 analyses is presented).

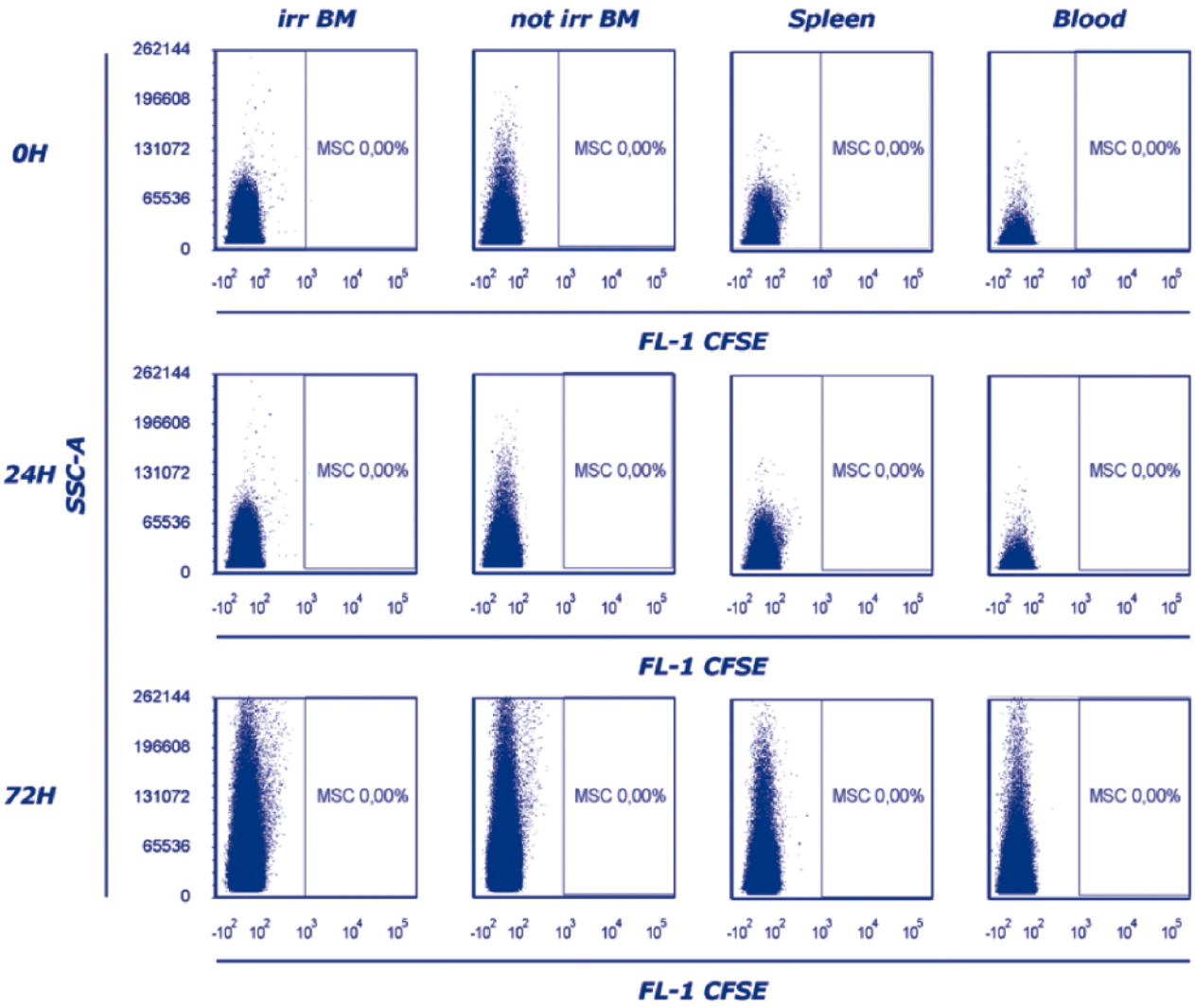
5.2 In vivo tracking of CFSE-labelled BM-MSC transplanted in locally irradiated BM

Six alloxan-induced diabetic C57BL/6 mice were sacrificed at 3 different time points after BM-MSC transplantation: immediately (n=2), 4 hours (n=2) and 24 hours (n=2). Immediately after transplantation mean 0.6% MSC CFSE+ were detected in the locally irradiated BM, mean 1,15% MSC CFSE+ in the non-irradiated BM, mean 0,0% MSC CFSE+ in the spleen and mean 0,0 MSC CFSE+ in the blood. At 4 hours after transplant mean 5,4% MSC CFSE+ were detected in the locally irradiated BM, mean 0,3% MSC CFSE+ in the non-irradiated BM, mean 0,0% MSC CFSE+ in the spleen and mean 0,0 MSC CFSE+ % in the blood. At 24 hours after transplant, mean 3,2% MSC CFSE+ were detected in the locally irradiated BM, mean 0,0% MSC CFSE+ in the non-irradiated, mean 0,0% MSC CFSE+ in the spleen and mean 0,1% MSC CFSE+ in the blood. This experiment shows that a small number of labelled BM-MSC can be detected. They are mainly located in the locally irradiated BM. At the time points analysed no BM-MSC were detected in the spleen or in the circulation. However, more numbers are required to draw conclusions about labelled BM-MSC survival in the BM (Fig 8).

5.3 In vivo tracking of CFSE-labelled BM-MSC co-transplanted with islets in locally irradiated BM

Six alloxan-induced diabetic C57BL/6 mice were sacrificed at 3 different time points after islets + BM-MSC infusion: immediately (n=2), 24 hours (n=2) and 72 hours (n=2) after the transplant. No labelled BM-MSC were detected at any time points in BM (irradiated and contralateral site), in spleen and in blood. This experiment demonstrates that adding islets in the BM does not facilitate BM-MSC engraftment (Fig 9).

Figure 9 -In vivo tracking of CFSE-labelled BM-MSC co-transplanted with islets in locally irradiated BM



Analysis of C57BL/6 mice transplanted with allogeneic islets and syngeneic MSC CFSE labelled cells 0-, 24-and 72-hours after transplantation. MSC CFSE+ are presented as a percentage of live cells (1 of the 2 analyses is presented).

Discussion

To improve engraftment and time to rejection, islets were co-transplanted with BM-MSC or pMSC into the BM. Our study is the first study to co-transplant BM-MSC or pMSC into the BM cavity. Our study demonstrates that both pMSC and BM-MSC enhance islets engraftment in the first days after transplantation. However it must be taken into account that the glycaemia levels before transplantation differ, which might influence the outcome.

Preclinical- and in vitro studies have already confirmed the beneficial effects on engraftment when islets are co- transplanted with BM-MSC[25,32,39-44]. One study has demonstrated that when pMSC are co-transplanted under the kidney capsule, they have the ability to facilitate normoglycaemia and neovascularisation[20]. MSC are appealing because they are easy to obtain and to expand[31] and have strong immune modulatory capacities. For instance MSC are known to have the ability to protect allogeneic islets by negatively regulating persistent effector T-cell[26], by preventing differentiation and maturation of DC[27], and by suppressing the activation and proliferation of B cells and the subsequent production of allo-antibodies[26]. Recently, our lab has identified a specific subtype of MSC, the pMSC20. In vitro and in vivo analyses show that pMSC have phenotypic, biologic and immunomodulatory characteristics similar to BM-MSC but they are not identical[20,32]. pMSC derive from the BM but reside in the pancreas and express negligible levels of islet-specific genes. Although pMSC do not have the potential to differentiate in β- cells they could exert an indirect role as helper cells and might have an extra beneficial effect on the micro-environment of the BM for the islets added to the known immune modulatory capacities of BM-MSC. However

our study did not find a beneficial effect of co-transplantation of pMSC over BM-MSC.

Although our experiments did not directly address the mechanisms of action, we might speculate that MSC can improve engraftment through vessel formation by differentiating into mature endothelial cells and by the secretion of proangiogenic factors[36], secretion of anti- inflammatory cytokines[37] and the release of growth factors[38]. All of these factors can directly assists in maintaining β-cell survival and function[25,33-35]. This effect is most likely to take place in the first days after transplantation[25,41,45,46] and is in accordance with our findings. In addition, engraftment in the early days after transplantation is important since it is estimated that 50-60% of the graft is lost early after intra-hepatic transplantation[10].

Despite a beneficial effect on islet engraftment in the first days after transplantation, we did not observe any advantages regarding allograft rejection. Strong proliferative T-cell responses, dominated by Th1 cytokines productions and high levels of cytotoxic T cells are associated with poor graft function[47,48]. T cells from C57BL/6 mice preferentially produce Th1 cytokines with high interferon-gamma (IFN-γ) and low IL-4 Th2 cytokines, which indicates that our model facilitates a strong alloreactive immune response[49]. On the contrary the absence of such T-cell responses or the predominance of anti-inflammatory cytokines is associated with persistent graft function[47]. Previous studies with MSC in islet transplantation have shown promising results on allograft rejection in the past[19,44,50,51]. That said, it should be noted that in these studies mainly BALB/c mice were being used as recipients. In future research the fierce immune response can be tempered by using other less stringent models or by immune suppressive drugs.

As previously mentioned our study did not find a beneficial effect of co-transplantion of pMSC over BM-MSC and therefore only BM-MSC were labelled with CFSE to determine whether they are able to remain in the BM after infusion. Splenocytes, BM-cells of both the locally irradiated-, and non-irradiated site and blood were analyzed. The experiments demonstrate that BM-MSC do not remain in the locally irradiated BM after adding islets. However, more numbers are required to draw conclusions about the capacity of islets to influence BM-MSC migration. In line with this result, a study by Iso Y. et al. reports that the effects on engraftment are generally without evidence that MSC reside in the injured tissue, suggesting that the favourable effects of MSC reflect the impact of transitory paracrine effects or secreted factors rather than engraftment, differentiation, or cell fusion[52]. Additionally, it is known that when BM-MSC are administered intravenously the majority of cells are entrapped in the lungs and just a minority migrates to the damaged tissue[53]. Although we transplanted the BM-MSC in the BM which is a extra-vascular site, the barrier is small and mainly for immature blood cells[54]. In addition, it is known that pMSC derive from the BM, but reside in the pancreas[20]. Thus, BM-MSC might escape into the systemic circulation and migrate to other tissues. This could explain why we were not able to detect BM-MSC after infusion. In order to overcome the problem of engraftment failure, avidin-biotin binding could be used to increase cell adhesion between BM-MSC and islets. In vitro, Neutravidin conjugated with biotinylated bsp-RGD(15) peptide provided the most robust cell adhesion[55]. It would be interesting to further explore this combination's potential for enhancing BM-MSC engraftment.

In conclusion, this study demonstrates a significant improved engraftment when islets are co-transplanted with BM-MSC into the locally irradiated BM compared to pMSC and islets alone. CFSE staining showed that no BM-MSC engraft in the BM and thus suggests that this favourable effect is most likely due to the impact of transitory paracrine effects rather than local effects. Although we found improved engraftment we were not able to postpone allograft rejection. This might be due to the stringent C57BL/6 mouse model, the migration of BM-MSC or other factors which need to be identified.

Acknowledgments

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Systematic Review

The association of prenatal exposure to paracetamol and neurodevelopmental disorders in childhood

A systematic review

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Abstract

Background: **Paracetamol is the most commonly used over-the-counter pain reliever and antipyretic among pregnant women. Paracetamol has been considered to be one of the safest analgesics, even for pregnant women. It is known that paracetamol crosses the placenta and that paracetamol and its metabolites enter the fetal blood flow. However, it is not yet well understood how this prenatal paracetamol exposure could affect the fetus. The aim of this review is to investigate what is known in the existing literature about prenatal exposure to paracetamol and child brain development, focusing on the development of Attention Deficity Hyperactivity Disorder/Hyperkinetic Disorder (ADHD/HKD) and Autism Spectrum Disorders (ASD).**

***Methods*:** **The PubMed database has been systematically searched for existing literature investigating prenatal paracetamol exposure and the effects on neurodevelopment in children.**

Results: **A total of 6 articles met the inclusion criteria. Two studies suggested that prenatal paracetamol use was associated with an increased risk for ADHD and HKD; one study showed an increased risk for ASD. Further, an ecological study showed a positive correlation between prenatal paracetamol exposure and the prevalence of autism. One study demonstrated an association between prenatal paracetamol exposure and different adverse developmental outcomes. Finally, there was one study that showed no association with child intelligence.**

Discussion: **This systematic review suggests that there is an association between prenatal exposure to paracetamol and an increased risk of neurodevelopmental disorders such as ADHD, HKD and ASD. More research on prenatal paracetamol use and the potential consequences on child development is needed before evidence-based recommendations can be developed.**

Introduction

Paracetamol, also known as acetaminophen, is the most commonly used over-the-counter pain reliever and fever reducer among pregnant women, with reported use between 65% to 75% in the USA and more than 50% in Europe[1]. The use of paracetamol during pregnancy increased after 1980, when evidence was found that use of prenatal salicylates, such as aspirin, was associated with Reyes syndrome[2,3]. Paracetamol has been considered one of the safest analgesics, even for pregnant women[4]. It is known that paracetamol crosses the placenta and that paracetamol and its metabolites enter the fetal blood flow[5]. However, it is not yet well understood how this prenatal paracetamol exposure could affect the fetus.

Worldwide, the brochure of paracetamol instructs consultation with the physician before taking the drug, that it is safe to breastfeed the child while taking paracetamol and that paracetamol is not recommended for children younger than 6 years. The Dutch brochure even states that paracetamol use during pregnancy is not harmful for the pregnancy or to the health of the unborn child.

However, recent animal and human studies have demonstrated delayed adverse effects of paracetamol. Prenatal paracetamol use has been shown to have endocrine-disrupting functions[6-9]. which increases the risk of cryptorchidism[10], as well as immune modulating characteristics, increasing the risk of asthma[11,12]. Moreover, high doses of paracetamol can also cause trauma to fetal liver cells, resulting in long term liver failure[13,14]. Even therapeutic doses of paracetamol have been proven to be harmful for the fetus since it may have important effects on (anti)oxidant balance[15,16]. There is no evidence for other adverse effects of paracetamol on birth outcomes, such as malformation, risk of miscarriage, low birth weight or prematurity[17]. However, Rebordosa et al. reported an association between prenatal paracetamol exposure and preterm birth in women with pre-eclampsia[18].

Hormones, (anti)oxidant balance and a regulated immune system are very important for brain development of the fetus. Thus maternal use of paracetamol during pregnancy could po-

tentially be related to neurological and behavioral disorders[19]. It has been suggested that the endocrine-disrupting functions of paracetamol could play a role in the development of Attention Deficit Hyperactivity Disorder (ADHD) and Hyperkinetic disorder (HKD)[20,21]. Moreover, it has also been suggested that maternal paracetamol use plays a role in the etiology of autism spectrum disorders (ASD) due to its immune modulating characteristics and disruption of the (anti)oxidant balance[12,22]. The prevalence of ADHD and HKD varies from 2% to 18% and 0.5 to 1% respectively. The global prevalence of ASD is estimated to be 7.6 per 1000. As the prevalence and incidence of these diseases haven been increasing since 1970, a good understanding of the underlying etiology and risk factors is important to prevent further exposure and development of these disorders.

The aim of this systematic review is to investigate in the existing literature about prenatal exposure to paracetamol and child brain development with a particular focus on the development of disorders including ADHD/HKD and ASD.

Methods

Search strategy

On January 6th 2016, the PubMed database has been systematically searched for English-language articles, using the following Medical Subject Headings (MeSH Terms): ((acetaminophen [MeSH Terms] OR analgesics[MeSH Terms] NOT narcotics[MeSH Terms]) AND (prenatal exposure delayed effects[MeSH Terms] OR prenatal[title/abstract] OR antenatal[title/abstract] OR perinatal[title/abstract] OR intrauterine[title/abstract] OR in utero[title/abstract] OR pregnancy[title/abstract] OR fetal[title/abstract]) AND humans[MeSH Terms]) AND (mental disorders [MeSH Terms] OR child behavior [MeSH Terms] OR behavioral symptoms [MeSH Terms] OR cognition [MeSH Terms] OR intelligence [title/abstract] OR IQ [title/abstract] OR neuropsychology [MeSH Terms]) OR ((acetaminophen [Title/abstract]) AND autism [Title/abstract] AND maternal [Title/abstract]). The last three terms (in italic) were added to the search to include a relevant article that was not included to the first search. All results were limited to human subjects.

Selection criteria

The articles were first screened by title and abstract. Articles without full text or inaccessible articles were excluded. Inclusion criteria were studies that concerned the use of paracetamol during pregnancy and the potential long-term effects that this prenatal exposure could have on childhood brain development in the broadest sense. Articles were excluded if they were case reports, reviews or a reply to articles. Articles that investigated the use of paracetamol during pregnancy and health problems other than alteration of brain development (e.g asthma) were also excluded. As the aim was to investigate the effect of prenatal paracetamol use on neurodevelopmental disorders in children only, a maximum age of 13 years was set on included subjects within the articles. Articles that met the inclusion criteria were read in full text.

Analysis

The primary outcome measure was the association between prenatal exposure to paracetamol and anomalies of the brain development in the offspring. An overview of the included articles is produced in Figure 1 and Table 1.

Results

Description of studies

Figure 1 shows that the systematic search in PubMed lead to 59 articles. One article has been published in December 2015 and has not yet been indexed in PubMed. Thus this article could not be found in the initial search in PubMed. To be able to include this article, a few Mesh Terms were added to the search (see Methods section) but it is expected that in a few months, this addition will not be required. After the first screening, 20 of the articles were potentially relevant. After the second screening, 6 articles remained to be included in the review (see Figure 1). The included studies are described in Table 1[23-28].

Figure 1 - Flow chart of study selection progress

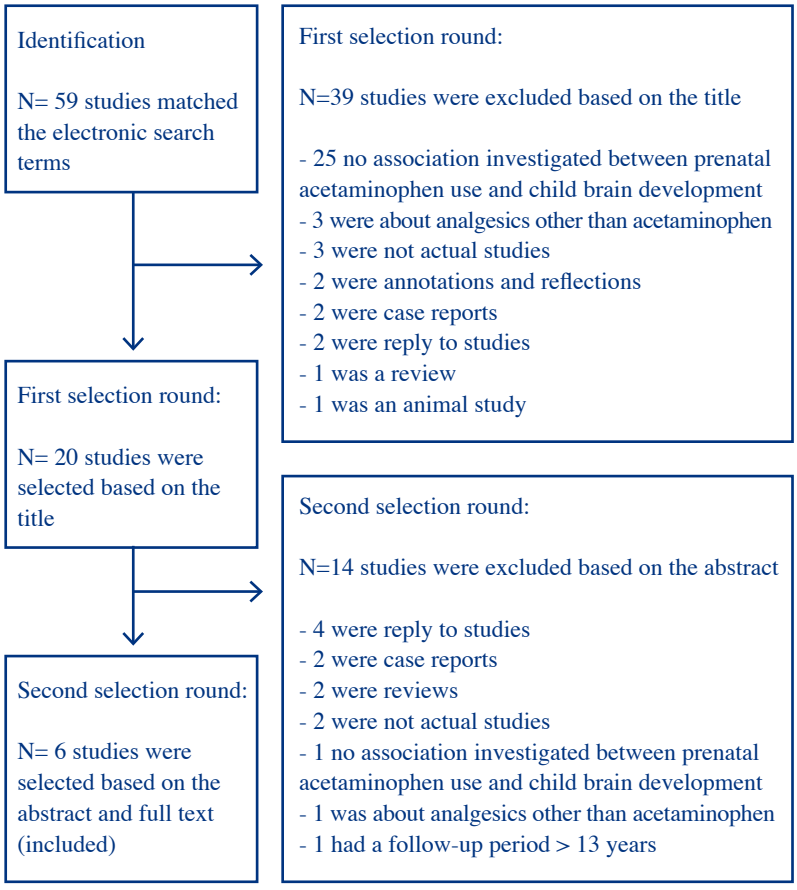

Table 1 - Characteristics and outcomes of included studies

Study	Population	Type study	Follow-up	(Assessment of) paracetamol use (%)	Tests used	Outcome
ADHD/HKD Liew Z et al. 2014	n= 64 322	Longitudinal population based prospective cohort study	13 years of follow-up	Three telephone interviews at 12 and 30 weeks gestation as well as 6 months postnatal (> 50%)	Strengths and Difficulties Questionnaire (SDQ) to measure symptoms of ADHD	Prenatal paracetamol use was related to an increased risk for ADHD-like behaviors
Thompson et al. 2014 ASD	n= 871	Longitudinal population based prospective cohort study	11 years of follow-up	Interviewer-administered questionnaires soon after giving birth (49.8%)	Strengths and Difficulties Questionnaire (SDQ) to measure symptoms of ADHD	Prenatal paracetamol use was associated with higher SDQ scores (ADHD symptoms)
Bauer & Kriebel 2013	n= 8 (countries)	Ecological analysis	-	Usage rates were extracted from studies examining the use of paracetamol during pregnancy	-	Prenatal paracetamol use was positively correlated with the prevalence of autism
Liew Z et al. 2015	n= 64 322	Longitudinal population based prospective cohort study	13 years of follow-up	Telephone interviews at 12 and 30 weeks of gestation, and 6 months postnatal (>50%)	International Classification of Diseases 10th Edition	Prenatal paracetamol exposure was associated with a higher risk for autism in children
Diverse developmental outcomes Brandlistuen et al. 2013	n= 22 418	Sibling-controlled prospective cohort study	3- years of follow-up	Two prenatal questionnaires and one postnatal (6 months) questionnaire (46.1%)	Ages and Stages Questionnaire (ASQ) to assess psychomotor development & Child Behavior Checklist to measure externalizing and internalizing behaviors	Prenatal paracetamol use was associated with adverse outcomes for gross motor and communication development, behavior and activity
Streissguth et al. 1987	n= 1529	Longitudinal population based prospective cohort study	4 years of follow-up	Self-report at 5 months gestation (43.5%)	Wechsler Preschool and Primary Scale of Intelligence (WPPSI)	Prenatal paracetamol use was not related to child IQ or attention

Subjects

The 6 studies were conducted in the Western world. The follow-up period varied from birth to 13 years. Five of these studies were cohorts. Most of the children were European.

In each study, the results were adjusted for several confounders, such as diseases or conditions that may trigger paracetamol use during pregnancy (fever, infections, inflammations), smoking and alcohol drinking during pregnancy, self-reported maternal psychiatric illnesses, concomitant use of any other medication, baseline characteristics of mother and child, including child's birth year, birth weight, sex, maternal age at child's birth, parity, gestational age at delivery and socioeconomic status. Maternal use of paracetamol during pregnancy was assessed using different methods, mostly using questionnaires or interviews via telephone. The prevalence of maternal paracetamol use during pregnancy varied between 43.5% and more than 50% in these studies.

ADHD/HKD

Two studies investigated the relation between prenatal paracetamol exposure and ADHD symptoms[24,26].

In Thompson et al.[24], symptoms of ADHD were assessed using the Strengths and Difficulties Questionnaire (SDQ). In this study, higher total difficulty scores were observed when paracetamol was used during pregnancy, but not if other drugs (e.g. antibiotics, analgesics) were used (parent SDQ at 7 (OR= 2.1; 95% CI 0.0-5.0), parent SDQ at 11 (OR= 1.2; 95% CI 0.6-2.5) and child SDQ at 11 (OR= 1.0; 95% CI 0.6-1.6)). Children of mothers who used paracetamol during pregnancy were also at increased risk of ADHD-problems at 7 and 11 years of age[26]. Particularly problematic were emotional and conduct problems at age 7[24].

In a Danish cohort, children's ADHD-like behaviors were assessed using the standardized Strengths and Difficulties Questionnaire (SDQ) and all HKD diagnoses were based on the International Statistical Classification of Diseases, 10th revision (F90.0-F90.9)[26]. Relying on the civil registration number, they also searched for children who used ADHD medications. An increased risk for ADHD-like behaviors was observed in children aged 7 years with maternal paracetamol use during pregnancy (RR, 1.13; 95% CI 1.01-1.27) as well as an increased risk for HKD diagnosis or ADHD medications. When women reported having used paracetamol for 20 weeks or more during

pregnancy, the risk for HKD diagnosis in children almost doubled (HR, 1.84; 95% CI, 1.39-2.45) and the risk of receiving ADHD medication increased by 50% (HR, 1,53; 95% CI 1,21-1.94). Thus, the risks increased with increasing frequency of paracetamol use throughout pregnancy. Correction for maternal use of ibuprofen and aspirin during pregnancy did not change the results, showing a specific effect of paracetamol.

ASD

Two studies examined the association between prenatal exposure to paracetamol and autism[23,27].

In the ecological study of Bauer and Kriebel in 2013, population weighted average autism prevalence rates and paracetamol usage rates were compared. They used the Autism Prevalence Summary Table which summarized the results of 59 prevalence studies conducted worldwide. In this study prenatal use of paracetamol was correlated with autism prevalence with a correlation of r=0.80[23]. Within the limits of the small datasets, the normality assumption was not seriously violated and so Pearson's parametric correlation coefficient was used with an information weighted (1/variance) linear regression model. Although a positive correlation between autism prevalence and indicators of prenatal paracetamol exposure were found, this ecological study did not address causation.

In Liew et al. in 2015, an ASD was assessed using the International Classification of Diseases 10th edition (ICD-10 F84.0-F84.9 for ASD). The association between maternal paracetamol use during pregnancy and offspring ASD diagnosis was investigated[27], using the same Danish cohort as the previous study of Liew et al. in 2014[26]. Their analysis suggested that prenatal exposure to paracetamol was associated with a higher risk for ASD and infantile autism in children. An increased risk for ASD and infantile autism was found if the mother reported paracetamol use during all three trimesters (HR, 1.39; 95% CI 1.14-1.70 and 1.49; 95% CI 1.07-2.07 respectively). The effect estimates for paracetamol use in pregnancy were stronger for ASD with hyperkinetic symptoms, and no associations were observed for ASD or infantile autism alone[27]. Maternal use of paracetamol during pregnancy was also associated with other subtypes of ASD, but only in those children with hyperkinetic symptoms. Effect estimates were similar in models including co-medication[27].

Diverse developmental outcomes

One sibling-controlled cohort study revealed that paracetamol use during pregnancy was associated with several adverse developmental outcomes[25]. Children prenatally exposed to paracetamol for more than 28 days had poorer gross motor development (β 0.24, 95% CI 0.12-0.51), poorer communication skills (β 0.20, 95% CI 0.01-0.39), increased externalizing behavior (β 0.28, 95% CI 0.15-0.42), internalizing behavior (β 0.14, 95% CI 0.01-0.28), and higher activity levels (β 0.24, 95% CI 0.11-0.38). The effect estimates were lower if the mother reported short-term (less than 27 days) paracetamol use. There was no association found between prenatal ibuprofen use and neurodevelopmental outcomes. This sibling-controlled analysis showed stronger effects than the cohort analysis of Liew et al[26,27].

Finally, the last study reported that maternal paracetamol use was not significantly related to child intelligence or attention variables at the age of 4 years (p= 0.48 and p= 0.28, respectively)[28].

Discussion

This systematic review suggests an association between prenatal exposure to paracetamol and ADHD, HKD and autism/ASD in young children. A brief discussion of each study used in this review will follow.

Two studies focused on the association between maternal paracetamol use and ADHD-like behavior of the child. Both studies showed a significant association and used reliable ADHD assessment data[24,26].

Thompson et al. analyzed possible confounding by multiple drugs use and showed specific effects of paracetamol on ADHD. In addition, the study showed a stronger association with ADHD when paracetamol was used to suppress fever. Liew et al. 2014 and Brandlistuen et al. suggested that the longer the paracetamol is used, the higher the risk of potential adverse effects.

Each study has its own limitations which can be complemented by the strengths. Strengths are the large sample size, the prospective design, the database used to detect ADHD and correction for important confounders such as co-medication, baseline characteristics of both mother and child, diseases or conditions that may trigger paracetamol use during pregnancy and smoking and alcohol use during pregnancy (these strengths apply for all studies mentioned in the current review, except the article of Bauer and Kriebel). These strengths make small effects detectable, limit maternal recall bias and are reliable for diagnosing disorders such as ASD and ADHD.

However, these two studies (Thompson et al. and Liew et al. 2014) also have similar limitations: they have a potential source of selection bias due to dropout, they did not have information on dosage of paracetamol use and the findings have to be limited to children of European ethnicity. The possibility of residual confounding or confounding by indication for these studies also exists as is often the case with observational studies.

Two studies focused on the association between maternal paracetamol use and ASD[23,27]. However, these studies are completely different and complement each other.

Liew et al. 2015 showed a significantly increased risk of ASD with hyperkinetic symptoms among children who were prenatally exposed to paracetamol. This association cannot be made for ASD alone. Together with the previous studies focusing on ADHD and HKD, it is more likely that paracetamol use causes hyperactivity, and not ASD. This study is also possibly residually confounded by indication, selection bias and genetic factors that could play a role in the etiology of ASD. This study cannot be generalized for ethnicities other than European ones and also did not have information about dosage of paracetamol use.

Bauer and Kriebel performed a different type of study, linking ecological trends to the prevalence of ASD. This ecological analysis showed a positive correlation between prenatal paracetamol use and ASD prevalence. This correlation is plausible, this ecological link cannot be used to infer causality. According to the authors, the study is possibly confounded and subjected to bias and misclassification. However, this study has the strength that it is generalizable.

Only Brandlistuen et al. focused on different neurodevelopmental outcomes. This sibling-controlled cohort study adjusted for familial confounding (which is a major strength of this study). The relation of prenatal paracetamol exposure and childhood neurodevelopment was stronger when comparing siblings. The outcomes in this study were too broad to be directly related to ADHD. Furthermore, as with the other studies, confounding by indication could be an issue. Also this study did not inform about dosage of paracetamol use and is only generalizable for European children.

Only Streissguth et al. focused on the influence of prenatal paracetamol use on the child intelligence, but the findings were not significant. However, this study only focused on paracetamol use in the first half of pregnancy, while the other studies mentioned that the third trimester is also an important period for brain development in children. Further, this study did not collect information about dosage of paracetamol use. Results of this study are only generalizable to the Northern-American population. This study is also subject to confounding by indication and like the other studies, does not predict causal relations.

In this systematic review, 6 articles were included, of which both studies of Liew et al. showed the strongest evidence for the effects of prenatal paracetamol exposure on the development of ADHD/HKD and possibly ASD. Brandlistuen et al. also provides strong evidence for adverse neurological outcomes after prenatal exposure to paracetamol, mostly because this study corrected for familial confounding. The ecological link of Bauer and Kriebel shows the least strong evidence, because it has only linked the paracetamol use trends to the ASD prevalence trends over time, without examining if this increased prevalence of ASD was found among children prenatally exposed to paracetamol. It is, however, striking that both trends follow each other.

Conclusion

Based on the existing literature, an association between prenatal exposure to paracetamol and a higher risk of neurodevelopmental disorders like ADHD and HKD has been suggested. This potential association also holds for ASD. Although the literature suggests a relation between prenatal exposure to paracetamol and childhood neurodevelopment, studies are sparse. More information on prenatal paracetamol use and the potential consequences on child development are needed before evidence based recommendations can be made.

Studies that further investigate the exact mechanisms of paracetamol and how these mechanisms are involved in the etiology of these neurodevelopmental disorders are essential. Studies should register the dosage of the paracetamol use and should adjust for several environmental factors that could influence neurodevelopment, such as timing, frequency, and duration of paracetamol use during pregnancy.

It is also suggested that other studies examining the effects of paracetamol in adolescence and adulthood should be reviewed. The current review only holds associations between prenatal paracetamol exposure and adverse neurodevelopmental outcomes in children up to 13 years, thus the conclusions of this review are not generalizable to other neurodevelopmental disorders that present later in lifetime, such as schizophrenia, psychotic symptoms, and depression.

A few studies have examined the relation between prenatal exposure to analgesics and schizophrenia/psychotic symptoms, but have not investigated paracetamol specifically.[29,30]

To move forward, observational studies should be improved. First, human studies could be combined with experimental animal studies to show a plausible causal relation. Next, observational cohort studies examining maternal paracetamol use during pregnancy could also be combined with investigating paternal paracetamol use in the same period in order to adjust for shared familial and genetic confounding. Further, minor improvements could be to include different ethnicities in the study population for generalizability. This is important because the worldwide paracetamol use during pregnancy is high.

In conclusion, further research is needed into the long-term neurodevelopmental effects of prenatal exposure to paracetamol to provide information in order to make evidence-based recommendations.

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Systematic Review

Dynamic CT Myocardial Perfusion Imaging: the new standard in detecting hemodynamically significant coronary stenosis?

A systematic review and meta-analysis

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Abstract

Objective: **the aim of this systematic review and meta-analysis is to assess the diagnostic properties of dynamic CT-myocardial perfusion imaging (CT-MPI), to identify flow-limiting coronary lesions. Fractional flow reserve (FFR) based on coronary angiography was considered the gold standard.**

Design: **we conducted a PubMed/Medline literature search to identify relevant manuscripts. Data on sensitivity, specificity and overall diagnostic accuracy (DA) of CT-MPI was then collected and a meta-analysis was performed. The used FFR cut-off value had to be between 0.74 and 0.81. Included studies had to be studies performed in Europe.**

Results**: four studies fulfilled the selection criteria, which included a total of 210 patients and 600 coronary arteries. Overall sensitivity and specificity were 0.89 (95% confidence interval [CI] 0.78 – 0.95) and 0.88 (95% CI 0.75 – 0.94), respectively. Overall DA was 0.88 (95% CI 0.85 – 0.91).**

Conclusions: **sensitivity, specificity and DA of dynamic CT-MPI are promising. Further research is necessary to proof our findings on a larger scale to recommend the use of this device in future daily clinical practice to detect hemodynamically significant stenosis.**

Introduction

In current clinical practice, coronary lesions can be adequately framed or ruled out by modern radiological modalities, including cardiac Computer Tomography (CT)[1]. However, using cardiac-CT as a method to determine whether a coronary stenosis is hemodynamically significant or not, is controversial[2-4]. Cardiac-CT can be used to detect coronary stenosis, but its' severity is often overestimated, which leads to unnecessary invasive coronary angiography (CAG)[2-4]. The gold standard in stipulating whether a stenosis is hemodynamically significant is CAG-based Fractional Flow Reserve (FFR)[5, 6]. Specificity of Cardiac-CT to identify FFR-positive lesions is only 50%[2-4]. A new promising way in detecting the functional gravity of a coronary stenosis is dynamic Computed Tomography Myocardial Perfusion Imaging (CT-MPI)[7]. In future, dynamic CT-MPI could possibly be added to coronary angiography [8] so that one non-invasive diagnostic test could provide an integral picture of a patients' coronary condition. This then may result in a reduction of invasive CAGs, which is beneficial for the patient. However, before these modalities are to be combined, the diagnostic value of dynamic CT-MPI has to be verified against the gold-standard FFR. Several low sample size diagnostic studies show good perspectives of implementing dynamic CT-MPI in daily clinical practice, but confirmation still has to be obtained by a large-scale investigation[8]. In this systematic review and meta-analysis we summarize and quantify the available evidence on the properties of CT-MPI to rule-in and rule-out FFR positive lesions.

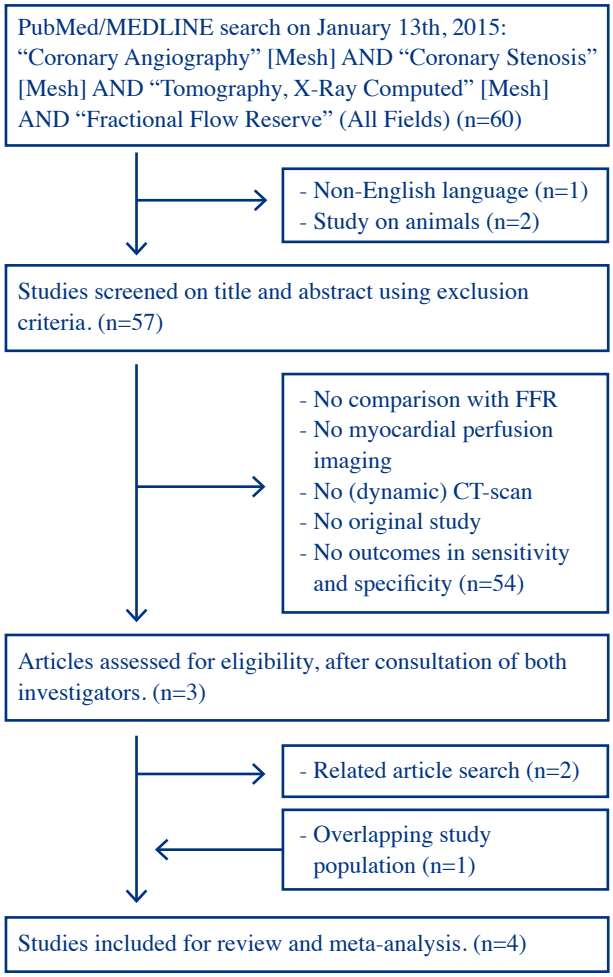
Methods

Literature search

On the 13th of January 2015 we searched PubMed/Medline for suitable studies, using keywords related to myocardial perfusion imaging, dynamic CT and FFR. The following search strategy was used: "Coronary Angiography"[Mesh] AND "Coronary Stenosis"[Mesh] AND "Tomography, X-Ray Computed"[Mesh] AND "Fractional Flow Reserve"[All Fields]. The search strategy was restricted to European articles written in English and limited to human subjects only. We excluded studies in which a) FFR was not used as the gold-standard, b) no myocardial perfusion imaging was performed, c) no (dynamic) CT-scan was used, d) no information was provided on sensitivity and specificity. Only original studies were included.

Titles and abstracts of all initially identified articles were independently reviewed by the two main investigators (TW and AE). Based on consensus, a reduced number of articles were

Figure 1- Flowchart of literature selection process



then selected for full reading. Besides, the related articles section on PubMed was screened for studies which could possibly be relevant to be included.

Data extraction

Sensitivity and specificity levels as mentioned in the included studies, were ran over by the investigators for obtaining numbers of True-Positive, False-Positive, True-Negative and False-Positive figures. Primary endpoint for our review was overall sensitivity and specificity, secondary endpoint was overall diagnostic accuracy (DA). DA was calculated according to the method of Alberg et al[9]. We collected data on sensitivity and specificity on coronary artery level, not on patient level.

Statistical analysis

OpenMeta[Analyst] open-source software was used to undertake meta-analysis. Forest-plots were constructed to demonstrate point estimates and corresponding 95% confidence intervals (CI) for the primary and secondary endpoints. Heterogeneity was assessed using Cochran's Q-statistic and I^2 values. A two-sided p-value < 0.05 was considered statistically significant for all tests.

Results

Search strategy

Our PubMed search produced 60 articles. After applying eligibility criteria, 57 articles remained for full reading, and 3 studies appeared suitable for inclusion. The additional search in the related article section in PubMed resulted in 2 additional articles that were considered suitable for inclusion. After full text reading, 1 article was excluded because of an overlapping study population. A flowchart illustrating the selection of articles in each stage of this systematic review is presented in figure 1.

Study Characteristics

Characteristics of the 4 included studies are presented in table 1. The sample sizes of the study populations used in the 4 studies ranged between 32 and 80, resulting in a total pool of 210 patients. Data on the study endpoints were available for altogether 600 coronary arteries. Three of the included studies (Bamberg, F., Huber, A.M. and Rossi, A. et al [7,11,12]) utilize a FFR cut-off value of 0.75. One study, (Greif, M. et al [8]), used a FFR cut-off value of 0.80. In all 4 included studies adenosine was used as the stress-inducing pharmacon during diagnostic testing. Sensitivity ranged from 76 to 95% and specificity from 75 to 100%.

There were 212 patients in total (72% men) with an average age of 65 years. All patients were suspected of coronary artery disease or had typical chest pain. The CT-scanning was performed specifically in the context of the studies.

Study endpoints

Based on the meta-analysis, the pooled overall sensitivity was 0.89 (95% CI 0.78 – 0.95) and an overall specificity of 0.88 (95% CI 0.75 – 0.94). Both sensitivity and specificity analyses showed

Table 1 - Characteristics of included studies

Author	Type of study	Number of patients	Number of vessels	Cut-off value FFR	CT-scanner	Stressor	Sensitivity (%, 95% CI)	Specificity (%, 95% CI)	DA (diagnostic accuracy)
Bamberg et al (2011)	Prospective feasibility study	33	99	≤0.75	Siemens second generation dual-source CT	Adenosine (0.14mg/kg/min)	93% (76-98%)	87% (76–93%)	88.5
Greif et al (2013)	Prospective, non-randomised, diagnostic study	65	195	≤0.80	Siemens second generation dual-source CT	Adenosine (0.14mg/kg/min)	95% (88-98%)	75% (65–83%)	85.1
Huber et al (2013)	Prospective study	32	96	≤0.75	Philips 256-section CT scanner	Adenosine (0.14mg/kg/min)	76% (57–87%)	100% (89–100%)	92.7
Rossi et al (2013)	Prospective, proof-of-principle study	80	210	≤0.75	Siemens second generation dual-source CT	Adenosine (0.14mg/kg/min)	88% (76–94%)	90% (85–94%)	89.5

Figure 2 - Forest plot of overall CT-MPI sensitivity

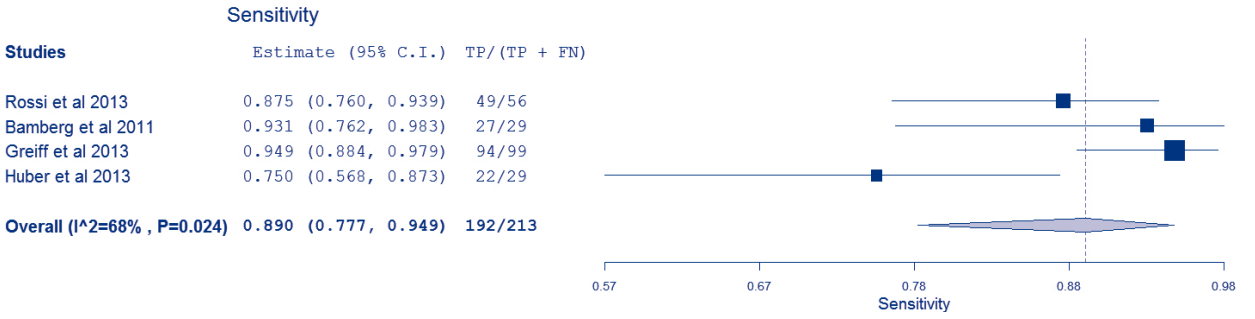


Figure 3 - Forest plot of overall CT-MPI specificity

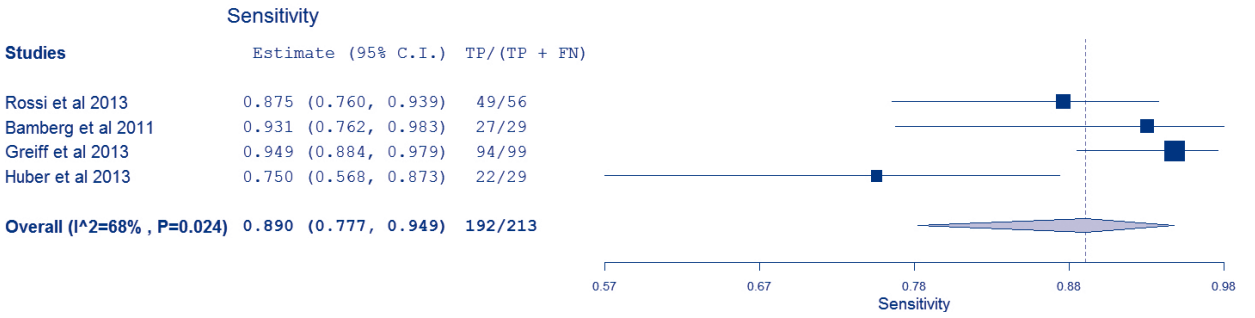
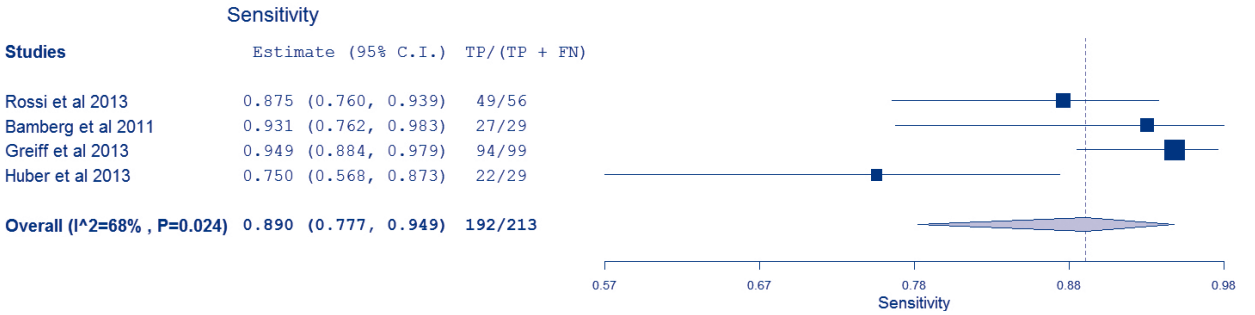


Figure 4 - Forest plot of overall CT-MPI Diagnostic Accuracy



significant heterogeneity (I^2 68% and 81%, respectively). The meta-analyses of overall sensitivity and specificity are shown in figure 2 and 3, respectively.

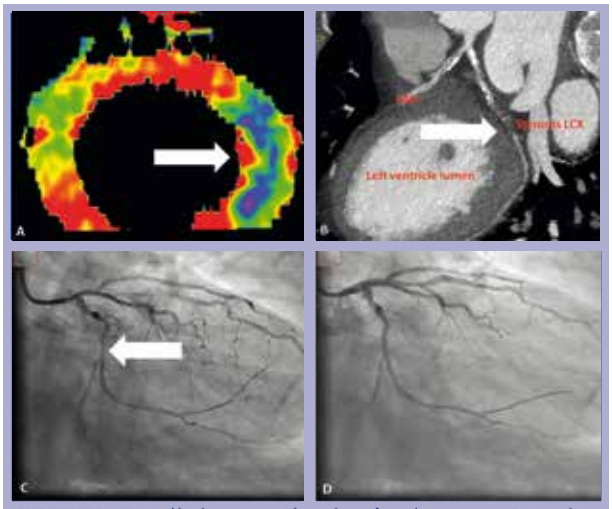
Considering our secondary outcome, a meta-analysis showed an overall DA of 0.88 (95% CI 0.85 – 0.91). The meta-analysis of the overall DA is shown in figure 4.

Discussion

The aim of our study was to investigate the diagnostic properties of CT-MPI to rule-in and rule-out flow limiting coronary stenosis by performing a systematic review and meta-analysis. Our study outcome suggests that combining computed tomography with angiography (CT-A) and myocardial perfusion imaging provides adequate diagnostic properties in detecting hemodynamically significant stenosis. Besides the satisfactory sensitivity, specificity and DA of dynamic CT-MPI, some other benefits for this diagnostic modality are applicable. This method is less invasive for patients, widely available and less costly compared to FFR-measurement[13,14]. Because of these benefits, in future, this diagnostic modality could possibly be used in daily clinical settings for detecting coronary stenosis.

Limitations

We observed significant heterogeneity in sensitivity and specificity, which means the presence of a true fluctuation between the effects of interest from the studie s involved[15]. This could be explained by the fact that Greif et al [12] used a different FFR cut-off value than all other studies used in our analysis. Also Huber et al [8] used a different CT-scanner for diagnostic testing. Even though all included studies used FFR measurements in classifying coronary artery stenosis, in most studies FFR measurement was only performed in vessels with an intermediate stenosis grade (between 30-90% diameter reduction on invasive angiography). As a result, not all included territories were confirmed FFR positive or negative for ischemia by an FFR pr essure wire.



CASE EXAMPLE: A 51 year old male presenting with complaints of typical angina pectoris. Present risk factors: hyperlipidemia, smoking and positive family history of cardiovascular disease. Bike exercise testing showed no signs of ischemia.

A) CT-perfusion scan showing a perfusion defect in the lateral territory associated with the left circumflex coronary artery (LCX). (white arrow)
B) A sub total stenosis of the LCX was seen on the CT angiography. (white arrow)
C) Invasive angiography showed a subtotal stenosis in the LCX (white arrow), hemodynamic significance was confirmed with an invasive FFR of 0.25.
D) After this FFR measurement, a successful stenting procedure was performed.

The CT-perfusion scan was made using a second generation dual source CT scanner. Radiation exposure for CT-angiography amounted 1.6mSiv, CT-perfusion 7.8mSiv.

Additional search

Two studies were found by searching related articles section on PubMed. One article (Kono et al [10]) was published very recently so the study wasn't yet assigned with MeSH-terms. This might explain why this article didn't show up in our original literature search. The study of Greif et al [8] matches with the MeSH-terms we used within our literature search, but it didn't appear in the list of results.

We decided to restrict our literature search to studies performed in Europe only. Within our literature search several non-European studies showed up, but these studies didn't met the inclusion criteria (e.g. by using a SPECT or PET-CT scanner as a myocardial perfusion imaging device). Furthermore, restricting our literature search to European articles only makes the results well applicable to European daily clinical practice.

Recommendation

Before dynamic CT-MPI could be used in daily clinical practice for detecting hemodynamically significant stenosis, we suggest to carry out a large multicenter study to confirm the comparability in diagnostic properties between CT-MPI and FFR. This future study should be carried out by using the same CT-scanner and FFR cut-off value in all participating centers.

Conclusion

Dynamic CT-MPI shows promising diagnostic properties in detecting hemodynamically significant stenosis, speaking in terms of sensitivity, specificity and DA. In future, this diagnostic modality could possibly be used in daily clinical practice. Before CT-MPI could definitively be marked as the new gold standard, further research is required to confirm the diagnostic value of CT-MPI.

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Percutaneous Coronary Intervention with Drug Eluting Stents versus Coronary Artery Bypass Graft Surgery for the Treatment of Unprotected Left Main Disease

A systematic review

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Abstract

Objective: **The aim of this systematic review was to compare the outcomes of coronary artery bypass graft surgery (CABG) versus percutaneous coronary intervention (PCI) with drug-eluting stents (DES) for unprotected left main coronary artery disease (ULMCAD).**

Methods: **We searched the online database Medline (PubMed) on January 9th, 2015 to find articles that compared CABG with PCI and DES for patients with ULMCAD in randomized controlled trials. Primary outcomes had to include one or more of the major adverse cardiac and cerebrovascular events (MACCE).**

Results: **Four studies published between 2008 and 2014 were included in the systematic review for a total of 1611 participants. For the outcomes MACCE and all-cause mortality, PCI seemed similar to CABG. Only for the subgroup of patients with a Syntax score ≥33, PCI appeared significantly worse. Repeated revascularization occurred more frequently in the PCI group.**

Conclusions**: CABG and PCI with DES are both viable options for the treatment of patients with ULMCAD in patients with low or intermediate Syntax scores. However, in complex lesions (high Syntax scores), CABG deserves the preference. Also, in the PCI group there is an increased revascularization rate overall.**

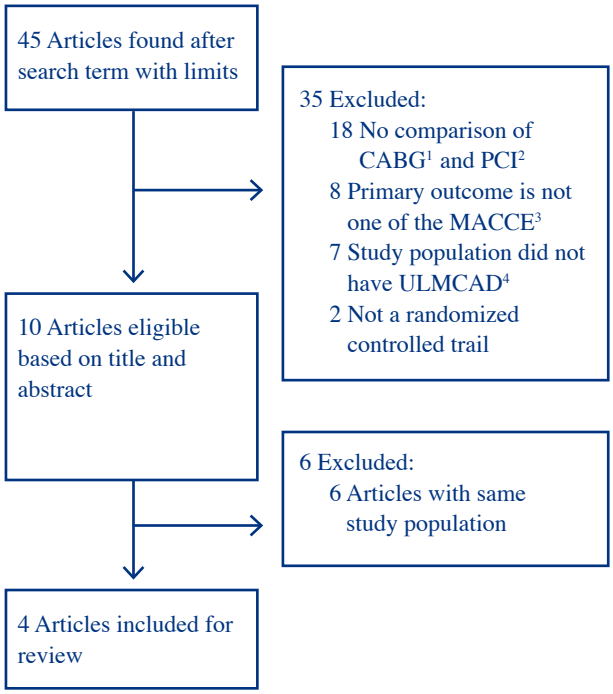
Introduction

Coronary artery bypass grafting (CABG) and percutaneous coronary intervention (PCI) are the revascularization treatment options for patients with unprotected left main coronary artery disease (ULMCAD)[1]. Since coronary artery disease (CAD) is still the leading cause of death globally[2], this is an important matter. The best treatment option for patients with left main stenosis is still not clear, while this specific group has a high mortality rate within the CAD population[3]. Previous versions of the guidelines stated that CABG was the golden standard for treatment of ULMCAD[4], but the most recent versions indicate PCI as a viable alternative for certain patients with ULMCAD. That is because PCI combined with placement of a drug-eluting stent (DES) has shown similar long-term clinical outcomes[5]. Both techniques have significantly improved over the last few decades[6]. The use of the internal mammary artery along with improved cardiopulmonary bypass has advanced the CABG procedure[7], whereas the introduction of DES has provided better results regarding restenosis and stent thrombosis after PCI [6]. However, the question remains which treatment leads to the best clinical outcome when using the currently available techniques. We therefore undertook a systematic review to compare the outcomes of CABG versus PCI with DES in patients with ULMCAD.

Methods

We conducted a systematic review of studies that compared CABG with PCI and DES for patients with ULMCAD. To find articles we performed a search on the online database Medline (PubMed) on January 9th, 2015. The search term used was: "Coronary Artery Bypass"[Mesh] AND ("Percutaneous Coronary Intervention"[Mesh] OR "angioplasty"[All Fields]) AND "left main"[All Fields]. We restricted the search by adding the limit option 'Randomised Controlled Trial' to only include RTC's.
For articles to be included, they had to compare CABG with PCI

Figure 1 - Flow Diagram of the articles included in the systematic review



1=Coronary Artery Bypass Graft. 2=Percutaneous Coronary Intervention
3=Major adverse cardiovascular cerebrovascular event. 4= Unprotected left main coronary artery disease

using DES for the specific population of patients with unprotected left main coronary artery stenosis. Also, primary outcomes had to include the composite endpoint 'major adverse cardiac and cerebrovascular events' (MACCE): consisting of death, stroke, myocardial infarction and repeated revascularization. Articles were excluded if their patient population was a subgroup of the study population in this review. Regarding that matter, if studies specifically analysed diabetics, women only, or participants with left main bifurcation disease, they were excluded. When two or more studies analysed the same population, we only included the one with the longest follow-up, as long as it analysed our specific population. The inclusion of studies was performed by both authors separately. Afterwards, they were compared for similarity.

We choose to analyse the outcomes MACCE, all-cause mortality and repeated revascularization to compare studies in this systematic review. We also analysed these endpoints for different Syntax scores and EuroSCOREs. The Syntax score indicates the complexity of the CAD[8] and the EuroSCORE indicates the predicted operative mortality for patients undergoing cardiac surgery [9].

Results

Our PubMed search initially produced 45 publications (figure 1). After reading the titles and abstracts, 10 relevant articles were left based on our inclusion criteria. Those articles were read in full text and after applying our exclusion criteria, 4 trials remained to be used in this review.

The characteristics of the studies are described in table 1 and 2. All articles were published between 2008 and 2014. A total of 1611 subjects were included, of whom 809 (50%) underwent PCI with stenting and 802 (50%) underwent CABG. The smallest study described 105 subjects [10], and the largest 705 [11]. Follow-up time varied from 1 year [10, 12] to 5 years [11]. No significant difference was found between Syntax scores of the PCI group and the CAGB group. The mean EuroSCORE in the studies varied from 2.4[12] to 3.9 [11]. No significant difference was found in the age of the PCI group compared to the CABG group.

Table 3 demonstrates the results of MACCE, all-cause mortality and the need for repeated revascularization after the given procedure for the different studies. In table 4 these results are shown for the different Syntax score subgroups. None of these studies show a significant difference in MACCE between PCI and CABG, with the exception of Morice et al.[11], which shows a significant hazard ratio (HR) for PCI compared with CABG (HR 1.78. p=0.003) for the group with a Syntax score ≥33.

For the outcome all-cause mortality, Morice et al. [11] showed a significant HR of 0.50 (p=0.02) for PCI compared with CABG in the subgroup with a Syntax score <33.

Boudroit et al. [12] used a non-inferiority margin of 7% to compare PCI to CABG. The 95% confidence interval for the difference between PCI and CABG all-cause mortality was -9.4 to 2.7, with a p<0.001 for non-inferiority.

Table 1 - Characteristics of the included studies – Part I

Study	Year	Country	n Included	n PCI	n CABG	Follow up time	Age PCI(yrs)	Age CABG(yrs)	p Value	Male PCI (%)	Male CABG (%)	p Value
Morice et al. (11)	2014	17 countries in Europe and United States	705	357	348	5 years	65.4±9.8	65.6±10.1	-	72.0	75.6	-
Boudroit et al. (12)	2011	Germany	201	100	101	1 year	66(62-73)	69(63-73)	0.24	72	78	0.49
Park et al. (13)	2011	South Korea	600	300	300	2 years	61.8±10.0	62.7±9.5	0.34	76	77	0.77
Buszman et al. (10)	2008	United States	105	52	53	1 year	60.6±10.5	61.3±8.4	0.69	60	73	0.13

PCI=Percutaneous Coronary Intervention, CABG=Coronary Artery Bypass Graft

Table 2 - Characteristics of the included studies – Part II

Study	Syntax PCI	Syntax CABG	p Value	EuroSCORE PCI	EuroSCORE CABG	p Value
Morice et al. (11)	29.6±13.5	30.2±12.7	-	3.9±2.8	3.9±2.9	-
Boudroit et al. (12)	24.0(19.0-29.0)	23.0(14.8-28.0)	0.09	2.4(1.5-3.7)	2.6(1.7-4.9)	0.08
Park et al. (13)	24.4±9.4	25.8±10.5	0.09	2.6±1.8	2.8±1.9	0.16
Buszman et al. (10)	25.2 ± 8.7	24.7 ± 6.8	0.75	3.3 ± 2.3	3.5 ± 2.3	0.65

PCI=Percutaneous Coronary Intervention, CABG=Coronary Artery Bypass Graft

Table 3 - Results in studies about the difference in risk for MACCE, all-cause mortality and repeat revascularization

Study	MACCE	p Value	All-cause mortality	p Value	Repeat revascularization	p Value
Morice et al. (11)	HR 1.23 (95% CI 0.95-1.59)	0.74	HR 0.88 (95% CI 0.58-1.32)	0.53	HR 1.82 (95% CI 1.28-2.57)	<0.001
Boudroit et al. (12)	95% CI for differences	0.19*	95% (CI for differences	<0.001*	95% CI for differences	0.35*
	-5.3-15.7		-9.4-2.7)		-0.3-17.1	
Park et al. (13)	HR 1.50 (95% CI 0.90-2.52)	0.12	HR 0.69 (95% CI 0.26-1.82)	0.45	HR 2.18 (95% CI 1.10-4.32)	0.02
Buszman et al. (10)	RR 1.09 (95% CI 0.85-1.38)	non significant	1.9% for PCI vs.	0.37	9.6% for PCI vs.	0.97
			7.5% for CABG		9.4% for CABG	

MACCE=Major Adverse Cardiac and Cerebrovascular Events, PCI=Percutaneous Coronary Intervention, CABG=Coronary Artery Bypass Graft, HR=Hazard Ratio, CI=Confidence Interval, RR=Relative Risk. HR's are for PCI in comparison with CABG.
*p Value for non-inferiority of 7% absolute difference

Table 4 - Results in studies about the difference in risk for MACCE, all-cause mortality and repeat revascularization for Syntax scored subgroups

Study	Syntax score	MACCE	p Value	All-cause mortality	p Value	Repeat revascularization	p Value
Morice MC et al. (11)	<33	HR 0.94 (95% CI 0.67-1.33)	0.74	HR 0.50 (95% CI 0.27-0.91)	0.02	HR 1.23 (95% CI 0.79-1.91)	0.36
	≥33	HR 1.78 (95% CI 1.21-2.63)	0.003	HR 1.59 (95% CI 0.90-2.83)	0.11	HR 3.30 (95% CI 1.86-5.88)	<0.001
Boudroit E et al. (12)	-	-	-	-	-	-	-
Park SJ et al.(13)	>29	HR 1.60 (95% CI 0.73-3.54)	0.24	-	-	-	-
	>19 to ≤29	HR 2.32 (95% CI 0.82-6.57)	0.11	-	-	-	-
	≤19	HR 1.38 (95% CI 0.40-4.21)	0.57	-	-	-	-
Buszman PE et al. (10)	-	-	-	-	-	-	-

MACCE=Major Adverse Cardiac and Cerebrovascular Events, PCI=Percutaneous Coronary Intervention, CABG=Coronary Artery Bypass Graft, HR=Hazard Ratio, CI=Confidence Interval, RR=Relative Risk. HR's are for PCI in comparison with CABG.

For the endpoint repeated revascularization, the trials by Morice et al. [11], and Park et al. [13] revealed significant HR's for PCI compared with CABG, respectively 1.82 (p<0.001) and 2.18 (p=0.02), in favour of CABG. The composite endpoint MACCE is mainly driven by rate of revascularization.

Discussion

CABG and PCI with DES are both viable options to consider for the treatment of patients with ULMCAD. Overall, PCI and CABG were similar regarding the clinical outcomes MACCE and all-cause mortality, but not for the need of repeated revascularization. In this respect, CABG seems to be superior. Also, when the lesions have a high Syntax scores, CABG appears to be significantly better with regards to MACCE and all-cause mortality. Therefore, CABG deserves the preference for complex lesions. PCI should be considered in all patients who have low or intermediate Syntax scores or a high surgical risk.

Not all studies showed the same results regarding the endpoints. Only Morice et al. [11] was consistent in revealing that CABG is significantly better than PCI. We believed that this was due to higher Syntax scores in this study population compared to the other studies. Also, this was a relatively large study which therefore had enough power to detect statistically significant differences.

Our review could be limited by the fact that the participants of the studies showed some heterogeneity in mean age, EuroSCORE and Syntax score, which could make them less comparable.

We recommend that future studies should focus on patient related factors such as comorbidity and surgical risk when comparing PCI with CABG. This will help selecting the best treatment option for the individual patient.

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- 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.

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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.

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