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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). We strive to release the journal twice a year. It is published on paper (750 copies) and on the EJM website (link below).

The main purpose EJM is to encourage medical and research master students to conduct research (empirical studies or systematic reviews), report on this research, and become acquainted with the 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 accepts 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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Ten years Erasmus Journal of Medicine

Dear reader, the first issue of the *Erasmus Journal of Medicine* was released back in 2010. Thus, to date, with this golden 14th issue, we celebrate the 10th anniversary of our *Journal*. As was the case with the previous issues, all articles are produced by Erasmus MC students. This issue is the first to welcome a contribution by one of our Nanobiology students. In the near future, articles might also be expected from our Bachelor students Clinical Technology, as well as Erasmus University College students who take the pre-med Life Science Major.

It is Huib Pols, former Dean of the Erasmus MC, who should have most credits for ten years *Erasmus Journal of Medicine*. Already in his inaugural lecture as professor in 1998 he stated: 'By writing research reports in the form of a scientific article, students are forced to think better about a good research question, the methods of research, and the ordering and interpretation of the obtained research results. The articles are then assessed according to the "peer review" concept within the faculty, and the best are ultimately published in the "Erasmus Student Journal of Medicine". [...] By sending the Journal to all students of our faculty, we can emphasize the importance of biomedical research.'[1]

As a Dean, in 2010, he was able to materialize his vision and he could proudly introduce the first issue that '[…] clearly elaborates on the versatility of our university medical center. You will be amazed. Do not just read the articles closely related to your field of study! Let the enthusiasm of all the authors who have contributed to this memorable first issue of the Erasmus Journal of Medicine get a hold on you.'[2] Also the current issue includes a broad spectrum of topics, ranging from lung-liver transplantation to next-generation sequencing in acute myeloid leukemia, and from treatment of right ventricular failure to an uncommon case of dysphagia. Take up and read!

We encourage our students to submit their assays, systematic reviews and, even better, their original research for publication in *Erasmus Journal of Medicine*. We also welcome student-editors and -reviewers. The link might not be causal, but most students that were involved in the first issue found their way as respected researchers in the international scientific community!

Prof. Maarten Frens, Vice-Dean Education

Prof. Eric Boersma, chair of the editorial board

[1] Prof. Huib Pols. De Arts-Onderzoeker: Op zoek naar een partner. Oratie. Rotterdam, 1998, p18. [Translation by EB]

[2] Prof. Huib Pols. Erasmus Journal of Medicine. 2010;1;6.

Erasmus Journal of Medicine

Editorial comment on 'The prevention of risky behavior through online challenges among young people'

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Online challenges, in which people are contested through the internet to perform a certain task or to conduct specific behavior. *Online challenges*, often posted on social networking or video platforms like YouTube, Instagram or Snapchat, are popular among children and adolescents, and also among young adults. Some challenges, which are shared for merely entertainment purposes, go viral (i.e. being quickly and widely spread on the internet by sharing on social media and e-mail), as people challenge each other to imitate the post and thus continue a chain of reactions that spreads widely. Sometimes challenges are so popular or dangerous that they are picked up by newspapers or news websites. For example, the Tide pod challenges, in which teenagers ingest laundry pods, which can cause severe burns to the mouth, respiratory tract or esophagus, vomiting, diarrhea, and seizure, and can lead to death [1, 2]. Or the choking challenge, an asphyxia game in which a person either strangles themselves or gets another person to strangle them in order to experience a brief euphoric feeling by depriving their brain of oxygen, also known as cerebral hypoxia [3, 4]. However, some challenges are not dangerous at all (e.g. bottle flip challenge, or mannequin challenge); are designed to raise money for charity ((ALS ice bucket challenge) or to make a positive contribution to the world (no makeup selfie challenge).

It is well-known that adolescents and young adults take more risks than any other age group [5, 6]. The question is 'why do adolescents engage in these dangerous challenges?', which can be generalized into 'why do adolescents in general engage in risky behavior? Examples of risky behavior include binge drinking, substance use, dangerous driving, performing illegal activities like vandalism, risky sexual behavior (e.g. unprotected intercourse or having multiple sexual partners) or participation in dangerous (online) challenges. First, risk taking and impulsivity observed in adolescents has been partly attributed to the slow development of brain regions necessary for cognitive control (e.g. inhibition), such as the prefrontal cortex [7]. In addition to the slow development of impulse control, adolescents also show a preference an immediate reward, which involves the dopaminergic reward system, which is activated by decisions involving immediately available monetary rewards, for example [8, 9]. Further, even when adolescents' behavior may have negative consequences, they seem to be biased toward potential rewards [7]. Thus, hypersensitivity of reward-processing regions together with the relatively slow neurodevelopment of impulse control regions has been proposed to account for increased risk-taking in adolescence, and particularly when decisions are made in an emotional context [10]. Finally, the social context is a particularly salient influence on adolescent decision-making; adolescents are especially prone to taking risks together with peers as the potential reward is peer approval [7, 11]. Thus, the answer on the question why adolescents engage in risky behavior is not straightforward, and involves numerous factors, including biological, psychological, and social factors [7].

In this edition of the Erasmus Journal of Medicine, Boer et al. aim to investigate the possibilities for prevention of participation in dangerous online challenges by young people based on a literature search, a survey with students, parents, teachers and experts (i.e. two pediatricians, a public health physician (in Dutch: GGD-arts) and the initiators of Foundation against internet abuse (in Dutch: Stichting T.I.M Tegen Internet Misstanden). In this paper, the researchers conclude that education, in particular by an (experience) expert combined with improved information for parents could contribute to prevention. First, from my point of view, this conclusion has been drawn to quickly. Intervention and prevention programs that have been evaluated and demonstrated to be effective in preventing (mental) health problems and risky behavior in adolescents and young adults are mostly based upon the best-available scientific evidence, rather than upon personal beliefs or anecdotal evidence. For example, the Iceland model, a multisectoral, community-based, collaborative system where researchers, policy makers, administrative leaders, and practitioners together with the local municipality, schools and parents join forces to reduce the odds of adolescent substance use over time, has proven to be effective [12]. Second, since the study of Boer et al. shows that the literature review yielded very limited evidence on the topic of (dangerous) online challenges, it should be clear that we need more high-quality multidisciplinary scientific studies at the intersection of epidemiology, psychology, neuroscience, social science and internet studies to explore a) the incidence and prevalence of participation in online challenges among children, adolescents, and young adults; b) the determinants of participation in online challenges and; c) the short and long-term consequences of participation in online challenges. In addition, more knowledge about what makes online challenges (harmful or not) go viral is needed. This knowledge will then increase our understanding of these new risky behaviors among children, adolescents and young adults, and inform public health practitioners and policymakers to develop effective evidence-based prevention programs.

References

1. *Valdez, A.L., et al., Pediatric exposure to laundry detergent pods. Pediatrics, 2014. 134(6): p. 1127-35.*
2. *Day, R., et al., Liquid laundry detergent capsules (PODS): a review of their composition and mechanisms of toxicity, and of the circumstances, routes, features, and management of exposure. Clin Toxicol (Phila), 2019. 57(11): p. 1053-1063.*
3. *Linkletter, M., K. Gordon, and J. Dooley, The choking game and YouTube: a dangerous combination. Clin Pediatr (Phila), 2010. 49(3): p. 274-9.*
4. *Andrew, T.A. and K.K. Fallon, Asphyxial games in children and adolescents. Am J Forensic Med Pathol, 2007. 28(4): p. 303-7.*
5. *Steinberg, L., A Social Neuroscience Perspective on Adolescent Risk-Taking. Dev Rev, 2008. 28(1): p. 78-106.*
6. *Kann, L., et al., Youth risk behavior surveillance--United States, 2013. MMWR Suppl, 2014. 63(4): p. 1-168.*
7. *Blakemore, S.J. and T.W. Robbins, Decision-making in the adolescent brain. Nat Neurosci, 2012. 15(9): p. 1184-91.*
8. *McClure, S.M., et al., Separate neural systems value immediate and delayed monetary rewards. Science, 2004. 306(5695): p. 503-7.*
9. *Kable, J.W. and P.W. Glimcher, The neural correlates of subjective value during intertemporal choice. Nat Neurosci, 2007. 10(12): p. 1625-33.*
10. *Somerville, L.H., R.M. Jones, and B.J. Casey, A time of change: behavioral and neural correlates of adolescent sensitivity to appetitive and aversive environmental cues. Brain Cogn, 2010. 72(1): p. 124-33.*
11. *Chein, J., et al., Peers increase adolescent risk taking by enhancing activity in the brain's reward circuitry. Dev Sci, 2011. 14(2): p. F1-10.*
12. *Sigfusdottir, I.D., et al., Prevention Is Possible: A Brief History of the Origin and Dissemination of the Icelandic Prevention Model. Health Promot Pract, 2020. 21(1): p. 58-61.*

The dilemma of the combined lung-liver transplantation sequence

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In this issue of the Erasmus Journal of Medicine, the systematic review of Hovius et al [1] assesses the difference in one-year survival rate between a combined lung-liver transplantation (LLTx) and single lung and liver transplantation. Combined lung/liver transplantation could be considered for patients with end-stage lung- and liver-disease. Interestingly, the LLTx has a lower one-year survival rate than the single lung and liver transplantations, respectively 75.2% and 80.0%, with a difference of 4.8%. However, no statistical analysis has been conducted on this difference in one-year survival. Because heterogeneity between the two groups can be present, these results should be interpreted cautiously.
One of the secondary outcomes of the meta-analysis by Hovius et al [1] was the effect of the transplantation sequence on one-year survival: liver-first or lung-first. Unfortunately, due to limited data from the included articles, no relevant conclusion could be drawn from the results.

One advantage of liver-first LLTx that is briefly mentioned by the authors is related to coagulopathy. Acute and chronic hepatic failure results in impaired haemostasis due to an imbalance between procoagulation and anticoagulation factor synthesis by the liver.[2] It is one of the main perioperative problems, causing bleeding tendency.[3,4] Intraoperative transfusions of red blood cells and plasma is shown to be associated with increased risk in mortality.[5] Also, coagulopathy is associated with an increased risk of primary graft dysfunction (PGD) of the lung and increased thromboembolic events.[6,7]
However, shortly after reperfusion of the liver transplant, the disrupted coagulability is able to recover.[8] One can speculate that after recovery of the coagulability through transplantation of the liver-first could improve outcome of a lung transplantation.

Although still a hypothesis, another possible advantage of transplanting the liver first is the immunological tolerance effect after liver transplantation. A review by Baroja-Mazo et al [9] illustrates processes how liver transplants show signs of allograft tolerance. Moreover, the transplanted liver can also protect other transplanted organs from immune-mediated graft damage and rejection, which is the case for combined liver-kidney and liver-heart transplantation.[10,11,12] Whether this induced systemic tolerance also affects the lung transplant and to what extent, could be a topic for future research.

As the present review by Hovius et al [1] already pointed out, a lung-first sequence is preferred when the lung will be exposed to an extended ischemic time. In practice, usually the organ in the worst condition gets transplanted first. No consensus has been reached on the preferable sequence of an LLTx. Nevertheless, it is a clinically relevant subject that should be investigated more.

References

1. *Hovius M, Van den Ban S, Jaspers E. A Meta-analysis of the One-Year Survival after Combined Lung-Liver Transplantation. Erasmus Journal of Medicine 2019.*
2. *Kujovich JL. Coagulopathy in liver disease: a balancing act. Hematology 2015(1):243-249.*
3. *Haagsma EB, Gips CH, Wesenhagen H, et al. Liver disease and its effect on haemostasis during liver transplantation. Liver 1985;5:123–12881.*
4. *Bechstein WO, Neuhaus P. Bleeding problems in liver surgery and liver transplantation. Chirurgie 2000;71:363–368.*
5. *Weber D, Cottini SR, Locher P, et al. Association of intraoperative transfusion of blood products with mortality in lung transplant recipients. Perioper Med (Lond). 2013 Sep 27;2(1):20*
6. *Suzuki Y, Cantu E, Christie JD. Primary graft dysfunction. Semin Respir Crit Care Med. 013 Jun;34(3):305-319.*
7. *Izbicki G, Bairey O, Shitrit D, et al. Increased thromboembolic events after lung transplantation. Chest 2006 Feb;129(2):412-6.*
8. *Kang YG, Martin DJ, Marquez J, et al. Intraoperative changes in blood coagulation and thrombelastographic monitoring in liver transplantation. Anesth Analg 1985 Sep;64(9):888-96.*
9. *Baroja-Mazo A, Revilla-Ruin B, Parrilla P, et al. Tolerance in liver transplantation: Biomarkers and clinical relevance. World J Gastroenterol 2016 Sep 14;22(34):7676-91.*
10. *Taner T, Gustafson MP, Hansen MJ, et al. Donor-specific hypo-responsiveness occurs in simultaneous liver-kidney transplant recipients after the first year. Kidney Int. 2018 Jun;93(6):1465-1474.*
11. *Rasmussen A, Davies HF, Jamieson NV, et al. Combined transplantation of liver and kidney from the same donor protects the kidney from rejection and improves kidney graft survival. Transplantation. 1995 Mar 27;59(6):919-21.*
12. *Wong TW, Gandhi MJ, Daly RC, et al. Liver Allograft Provides Immunoprotection for the Cardiac Allograft in Combined Heart-Liver Transplantation. Am J Transplant. 2016 Dec;16(12):3522-3531.*

Epidural Analgesia
a painless delivery for all?

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Introduction

Although many women reflect upon their birth experience with contentment, 16.5% of women look back on their delivery negatively due to pain during labor.[1] Fitting pain relief may improve women's satisfaction regarding giving birth. In the Netherlands, the most recent directive concerning the treatment of pain during labor dates from 2008 and states that, on request, every woman in labor must receive adequate treatment of pain.[2] Epidural analgesia is delineated as first-line treatment, but an epidural has to be administered in a hospital by an anesthesiologist.[2] In 2017, 163.826 women gave birth in the Netherlands, of whom 117.155 did so in the hospital.[3] In total, only 33.643 women received epidural analgesia, which exhibits that epidural analgesia is not given routinely, even when labor takes place in the hospital.[3] One could argue that the directive concerning pain relief during labor is in need of modernization, for it is questionable whether an on request-policy results in the highest achievable satisfaction among women in labor. Perhaps, pain relief should be routinely offered to all women giving birth in the hospital. This way, the option to get an epidural will shift from no, unless… as starting point in the treatment of labor pain to yes, unless… and pain during labor will be alleviated to a higher degree. However, can we take side effects and complications that may arise for granted? Does the pain relief that is achieved by routinely offering analgesia outweigh disadvantages such as adverse effects, making it a good alternative to an on request-policy?

This article investigates the consequences of a yes, unless… approach by reviewing literature on the different possibilities and consequences of pain relief, in particular epidural analgesia. Subsequently, these consequences are weighed, using the principles of biomedical ethics as defined by Beauchamp and Childress, for these principles are considered to be able to explain all essential aspects of medical ethics.[4]

Pain relief during labor: the facts

There are different ways to alleviate pain during labor. An extensive systematic review inquiring interventions to reduce labor pain did not find evidence, or only very limited evidence, of the effectiveness of various non-pharmacological interventions, such as acupuncture and hypnosis, and medicaments such as parenteral opioids and NSAIDs.[5] Epidural analgesia, and, to a lesser extent, inhaled analgesia, are the only effective methods to manage pain during labor.[5] Epidural analgesia is carried out by administration of a local anesthetic in the epidural space using a needle or catheter.[6] Different methods of administration and dosage show similar outcomes, in terms of pain relief, length of labor, mode of delivery, side effects and neonatal outcomes.[7] Although in an extensive database an overall (initial) failure rate of obstetric epidural analgesia of 14% was observed, ultimately almost all women received adequate pain relief.[8] A recent study and a systematic review, both comparing epidural analgesia to other methods of pain relief, also demonstrated epidural analgesia to be the most effective technique to diminish labor pain and improve women's satisfaction.[9,10] Moreover, epidural analgesia has been shown to lower stress levels during and immediately after delivery [11] as well as the risk of maternal depression 6 weeks and 2 years after giving birth.[12]

However, the use of epidural analgesia is not risk-free. Possible adverse effects of epidural analgesia are hypotension, itching, urine retention, fever, short motor blockade and a prolonged duration of delivery.[7,10,13,14] Epidural analgesia could also lead to spinal hematoma or abscess, however, this occurs very seldom: in a study that examined over 2.3 million procedures of obstetric epidural analgesia in the USA between 1998 and 2010, zero abscesses and 15 hematomas were observed (making the probability of a hematoma per procedure 1 in 154.730).[14] The use of epidural analgesia does not lead to an increase in neonatal adverse effects.[10] Utilization of epidural analgesia can cause a rise of assisted vaginal births. However, this side effect was only observed in studies conducted before 2005.[10] In a large Dutch cohort of child births between the year 2000 and 2009 an increase in unplanned caesarean sections was shown to be associated with the use of epidural analgesia, but, likewise, this correlation weakened over time.[15] The modernization of techniques used to administer epidurals and the diminishing concentrations of local anesthetic that are given have been coined as possible explanations for these findings, as is the less restrictive use of epidural analgesia, which leads to less selection of women who already have more severe pain and obstetrical complications.[10,15]

Whether the epidural was administered early (mostly defined as less than 4 centimeters cervical dilatation) or late during labor did not influence neonatal outcomes and mode of delivery.[16] A Dutch randomized trial that compared outcomes of routinely offered epidural analgesia to outcomes of analgesia on request shows that routinely offering epidural analgesia does not lead to a higher quality of life of the mother six weeks after childbirth.[17] Of the women who were offered epidural analgesia routinely, 89.3% ultimately received an epidural, as opposed to 47% of women when an on request-policy was followed and epidural analgesia was not actively offered.[17] Hypotension and motor block were more likely to arise in the routine group and a routinely offer-policy is thought to lead to more operative deliveries compared to an on request-policy.[18]

The preference to receive epidural analgesia is positively associated with age, positive attitude of caregivers to epidural analgesia and a diminished ability to cope with pain.[19] In addition to that the desire to deliver free of pain and the presence of acquaintances with positive experiences both influence the choice of epidural analgesia positively, whereas having fear of adverse effects influences this choice negatively.[20] The preference for epidural analgesia also is positively affected by nulliparity.[20,21] Fear may lead to more pain during delivery.[22,23] Continuous support by caregivers can improve the experience of women in labor and diminish the need of caesarean sections, instrumental birth and the use of analgesia.[24]

There are major regional differences in the use of pain relief during labor. Administration rates of epidural analgesia in Dutch hospitals vary from 3% to 43% of deliveries [25] and vast variations in the use of pain relief can also be observed between Dutch provinces.[26] Of all Dutch hospitals, 3% never administers epidural analgesia.[13]

Ethical considerations

Epidural analgesia is the most effective form of pain relief during labor, but routinely offering epidurals leads to more adverse effects.[5,10] It remains to be seen whether a standard offer of epidural analgesia can lead to a gain in pain relief that outweighs all disadvantages.

Pain is an unpleasant experience, so enabling and proactively offering pain relief that is as optimal as possible will diminish trouble for the woman in labor. The principle of beneficence demands the physician to treat the patient well and to act in the best interest of the patient. Epidural analgesia, being the most effective method to treat labor pain,[5,10] is appropriate for this endeavor. In the Dutch directive concerning pain relief during delivery, as well as in a WHO directive, epidural analgesia is mentioned as first-choice treatment for labor pain.[2,27] Nevertheless, there are no indications that routinely offering an epidural will lead to a higher quality of life as compared to an on request-policy.[17]

The woman giving birth can be harmed by the adverse effects occurring due to the use of epidural analgesia. These effects mainly influence the mother-to-be; outcomes of the neonate seem to be unaffected.[10] Serious adverse effects may occur due to epidural analgesia, but they only seldom take place.[14] It is important to note that other (less effective) treatments of labor pain also carry side effects.[5] Opioids and inhaled analgesia for example may both lead to nausea, vomiting and drowsiness.[5,28,29] In the Netherlands, after epidural analgesia, opioids are the most frequently used pain relief during labor,[3] but even an extensive systematic review (which examined 70 studies) could only conclude that there was insufficient evidence to establish the safety of obstetric opioids.[28] Opioids are therefore not an adequate alternative to epidural analgesia. The reason that opioids, such as remifentanil, are still used in Dutch obstetrics is that some doctors utilize remifentanil as alternative when an epidural is contraindicated or to meet the desires of the woman in labor.[25,30]

The principle of nonmaleficence, i.e. the aim of physicians not to harm patients, requires careful consideration of adverse effects versus pain relief. Whether the administration of an epidural is proportional depends foremost on the severity of the labor pain and to what extent the potential side effects would be acceptable for the woman. Only the woman giving birth can assess how severe her pain actually is. Therefore, it is difficult for the physician to estimate if an epidural would be proportional, without taking the experience and preferences of the woman in labor into account. Thus, it is pivotal that the physician and mother-to-be talk about the pain that could arise during delivery. Ultimately, the woman in labor decides whether or not she wants an epidural, even when it is routinely offered: her choice must be respected. First providing information and then giving the patient opportunity to consider as well as to reflect on the different aspects of the options, in the end enabling her to come to an own decision is known as shared decision-making.[31] However, current literature regarding the topic of decision-making ability cannot indisputably answer the question whether women who are going through excruciating pain still have sufficient decision-making ability to weigh the advantages and disadvantages of analgesia.[32] To mitigate this potential problem and to inform the pregnant woman as fully as possible, thorough counselling regarding the possibilities and impossibilities of epidural analgesia is needed before the beginning of labor. A recent article regarding the experiences of women in labor receiving pain relief also emphasizes the importance of giving pregnant women complete information concerning benefits as well as risks of various ways to alleviate pain.[33] This way, through shared decision making, women can choose the method of pain relief deemed most fitting. If, for example, epidural analgesia is preferred, informed consent must be obtained. In a Dutch study assessing the procedure of administering epidural analgesia it was observed that most of the times informed consent regarding epidural analgesia was obtained prior to delivery.[13] Still, all possible side effects were hardly ever addressed.[13] The decision regarding method of pain relief is, when made prior to labor, not necessarily representative for the actual requests of the woman giving birth. More than half of the women who prior to labor stated they did not want to receive an epidural, changed their mind while giving birth and asked for an epidural nonetheless.[34,35] Discussing the possible shift of preferences during delivery should therefore be part of a prepartum consult. This consult could, for example, take place during week 30 of pregnancy. The directive 'Spontaneous Vaginal Birth' states that in this week a so-called 'plan of labor' should be drawn up, in which the availability of epidural analgesia is considered.[36] Conceivably, during this consult epidural analgesia can be discussed even more extensively, and, if the mother-to-be prefers epidural analgesia, informed consent can be obtained.

If epidural analgesia would be offered routinely during all labors taking place in the hospital, there would be no changes for women who would rather give birth at home. Referrals from midwife to gynecologist, sometimes even during delivery, often stem from requests for pain relief,[37-39] which will still be possible if the epidural is offered routinely. A standard offer, or at least a revision of the current directive for treatment of labor pain, could diminish regional variations in the use of treatment of pain.

Conclusion and recommendation

Routinely offering epidural analgesia to all women delivering in the hospital aims at diminishing pain during labor as optimal as possible. The relief that epidural analgesia can give outweighs the possible side effects that could occur. However, this does not necessarily mean that a standard offer is justified. The consideration of advantages and disadvantages is different for all laboring women, for each woman experiences and handles labor pain in her own way. Routinely offering epidurals disregards these variations. Epidural analgesia is the most effective treatment of labor pain, but not necessarily the most fitting for each woman. Thus, yes, unless… will lead to overtreatment, whereas no, unless… will not achieve optimal pain relief. I therefore propose to give each pregnant woman, irrespective of her preferences regarding place of birth, explicit information about the various possibilities of the treatment of labor pain. Only offering epidural analgesia would steer the pregnant woman inordinately in the direction of epidural analgesia. However, the choice regarding pain relief should be based on the preferences of the pregnant woman and she can only make a proper consideration if she is fully informed. Thus, among others, epidural analgesia should be discussed as most effective method and possible side effects must be mentioned. Such an informational consult should take place before delivery. The caregiver and pregnant woman should also go through factors that could influence the choice of the method of pain relief. It is important for the mother-to-be to make an informed consideration and to choose, if any, the method of pain relief that she deems most fitting. Epidural analgesia should thus not be routinely offered during all childbirths in the hospital, but the possibilities of analgesia during labor should be routinely and explicitly discussed before the commencement of delivery. This guarantees that the pregnant woman herself, informed by the physician, can weigh the benefits that an epidural can give her, whilst being aware of the possible disadvantages. Hopefully, this way even more women will reflect upon their delivery positively.

References

1. *Rijnders M., Baston H., Schonbeck Y., et al. Perinatal factors related to negative or positive recall of birth experience in women 3 years postpartum in the Netherlands. Birth. 2008; 35: 107-116.*
2. *Nederlandse Vereniging voor Anesthesiologie en Nederlandse Vereniging voor Obstetrie en Gynaecologie. Medicamenteuze pijnbehandeling tijdens de bevalling. 2008;*
3. *Perined Perinatale Zorg in Nederland 2017. In Utrecht: Perined; 2019;*
4. *Beauchamp T.L., Childress J.F. Principles of Biomedical Ethics. New York: Oxford University Press, 2001.*
5. *Jones L., Othman M., Dowswell T., et al. Pain management for women in labour: an overview of systematic reviews. Cochrane Database Syst Rev. 2012: CD009234.*
6. *Silva M., Halpern S.H. Epidural analgesia for labor: Current techniques. Local Reg Anesth. 2010; 3: 143-153.*
7. *Gizzo S., Noventa M., Fagherazzi S., et al. Update on best available options in obstetrics anaesthesia: perinatal outcomes, side effects and maternal satisfaction. Fifteen years systematic literature review. Arch Gynecol Obstet. 2014; 290: 21-34.*
8. *Pan P.H., Bogard T.D., Owen M.D. Incidence and characteristics of failures in obstetric neuraxial analgesia and anesthesia: a retrospective analysis of 19,259 deliveries. Int J Obstet Anesth. 2004; 13: 227-233.*
9. *Czech I., Fuchs P., Fuchs A., et al. Pharmacological and Non-Pharmacological Methods of Labour Pain Relief-Establishment of Effectiveness and Comparison. Int J Environ Res Public Health. 2018; 15:*
10. *Anim-Somuah M., Smyth R.M., Cyna A.M., et al. Epidural versus non-epidural or no analgesia for pain management in labour. Cochrane Database Syst Rev. 2018; 5: CD000331.*
11. *Miller N., Asali A.A., Agassi-Zaitler M., et al. Physiological and psychological stress responses to labor and delivery as expressed by salivary cortisol: a prospective study. Am J Obstet Gynecol. 2019; 221: 351 e351-351 e357.*
12. *Liu Z.H., He S.T., Deng C.M., et al. Neuraxial labour analgesia is associated with a reduced risk of maternal depression at 2 years after childbirth: A multicentre, prospective, longitudinal study. Eur J Anaesthesiol. 2019; 36: 745-754.*
13. *Bos E.M.E., Schut M.E., de Quelerij M., et al. Trends in practice and safety measures of epidural analgesia: Report of a national survey. Acta Anaesthesiol Scand. 2018; 62: 1466-1472.*
14. *Rosero E.B., Joshi G.P. Nationwide incidence of serious complications of epidural analgesia in the United States. Acta Anaesthesiol Scand. 2016; 60: 810-820.*
15. *Wassen M.M., Hukkelhoven C.W., Scheepers H.C., et al. Epidural analgesia and operative delivery: a ten-year population-based cohort study in The Netherlands. Eur J Obstet Gynecol Reprod Biol. 2014; 183: 125-131.*
16. *Sng B.L., Leong W.L., Zeng Y., et al. Early versus late initiation of epidural analgesia for labour. Cochrane Database Syst Rev. 2014: CD007238.*
17. *van den Bosch A.A.S., Goossens M., Bonouvrie K., et al. Maternal quality of life in routine labor epidural analgesia versus labor analgesia on request: results of a randomized trial. Qual Life Res. 2018; 27: 2027-2033.*
18. *Wassen M.M., Smits L.J., Scheepers H.C., et al. Routine labour epidural analgesia versus labour analgesia on request: a randomised non-inferiority trial. BJOG. 2015; 122: 344-350.*
19. *Wassen M., Miggiels L., Devlieger R., et al. Women's prelabour preference for epidural analgesia: a cross-sectional study among women from the Netherlands and Belgium. J Psychosom Obstet Gynaecol. 2013; 34: 22-28.*
20. *Van den Bussche E., Crombez G., Eccleston C., et al. Why women prefer epidural analgesia during childbirth: the role of beliefs about epidural analgesia and pain catastrophizing. Eur J Pain. 2007; 11: 275-282.*
21. *Haines H.M., Rubertsson C., Pallant J.F., et al. The influence of women's fear, attitudes and beliefs of childbirth on mode and experience of birth. BMC Pregnancy Childbirth. 2012; 12: 55.*
22. *Saisto T., Kaaja R., Ylikorkala O., et al. Reduced pain tolerance during and after pregnancy in women suffering from fear of labor. Pain. 2001; 93: 123-127.*
23. *Logtenberg S.L.M., Verhoeven C.J., Rengerink K.O., et al. Pharmacological pain relief and fear of childbirth in low risk women; secondary analysis of the R AVEL study. BMC Pregnancy Childbirth. 2018; 18: 347.*
24. *Bohren M.A., Hofmeyr G.J., Sakala C., et al. Continuous support for women during childbirth. Cochrane Database Syst Rev. 2017; 7: CD003766.*
25. *Freeman L.M., Dahan A., van Lith J.M.M., et al. Epidural analgesia versus remifentanil patient controlled analgesia in labor: a survey of practice in the Netherlands. Int J Clin Anesthesiol. 2016; 4: 1051.*
26. *Seijmonsbergen-Schermers A.E., Zondag D.C., Nieuwenhuijze M., et al. Regional variations in childbirth interventions in the Netherlands: a nationwide explorative study. BMC Pregnancy Childbirth. 2018; 18: 192.*

27. *WHO Reproductive Health Library. WHO recommendation on epidural analgesia for pain relief during labour. Geneva: World Health Organization; 2018;*
28. *Smith L.A., Burns E., Cuthbert A. Parenteral opioids for maternal pain management in labour. Cochrane Database Syst Rev. 2018; 6: CD007396.*
29. *Phillips S.N., Fernando R., Girard T. Parenteral opioid analgesia: Does it still have a role? Best Pract Res Clin Anaesthesiol. 2017; 31: 3-14.*
30. *Logtenberg S.L.M., Vink M.L., Godfried M., et al. [Practice variation in the application of remifentanil during labour; an overview of its application in Dutch hospitals] Praktijkvariatie in het gebruik van remifentanil tijdens de partus. Ned Tijdschr Geneeskd. 2018; 162:*
31. *Broersen S. Shared decision making voor beginners. In: Medisch Contact; 2011;*
32. *Wada K., Charland L.C., Bellingham G. Can women in labor give informed consent to epidural analgesia? Bioethics. 2018:*
33. *Thomson G., Feeley C., Moran V.H., et al. Women's experiences of pharmacological and non-pharmacological pain relief methods for labour and childbirth: a qualitative systematic review. Reprod Health. 2019; 16: 71.*
34. *Pennell A., Salo-Coombs V., Herring A., et al. Anesthesia and analgesia-related preferences and outcomes of women who have birth plans. J Midwifery Womens Health. 2011; 56: 376-381.*
35. *Kpea L., Bonnet M.P., Le Ray C., et al. Initial Preference for Labor Without Neuraxial Analgesia and Actual Use: Results from a National Survey in France. Anesth Analg. 2015; 121: 759-766.*
36. *Nederlandse Vereniging voor Obstetrie en Gynaecologie. NVOG-richtlijn Spontane vaginale baring. 2013;*
37. *Offerhaus P.M., Geerts C., de Jonge A., et al. Variation in referrals to secondary obstetrician-led care among primary midwifery care practices in the Netherlands: a nationwide cohort study. BMC Pregnancy Childbirth. 2015; 15: 42.*
38. *Offerhaus P.M., Hukkelhoven C.W., de Jonge A., et al. Persisting rise in referrals during labor in primary midwife-led care in the Netherlands. Birth. 2013; 40: 192-201.*
39. *Amelink-Verburg M.P., Verloove-Vanhorick S.P., Hakkenberg R.M., et al. Evaluation of 280,000 cases in Dutch midwifery practices: a descriptive study. BJOG. 2008; 115: 570-578.*

Advances in microfluidic approaches for the detection and isolation of circulating tumour cells

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Abstract

Circulating tumour cells (CTCs) are mobile tumour cells that circulate in the peripheral blood and have the potential to invade and colonize tissues that constitute and express all factors essential to mediate metastasis. CTCs have gained attention as promising biomarkers for early diagnosis of cancer, as well as for making a validated assessment of anti-tumour activity of (precision) treatments. This source of information can be attained by means of a non-invasive 'liquid-biopsy', further aiding in the popularity of using CTCs for cancer characterization. The field of CTC research has undergone a significant increase in popularity and many novel detection methods have been developed. The main challenge that must be addressed is the scarcity of CTCs that circulate in the blood, which ultimately results in the necessity of CTC enrichment before analysis can be performed. Over the past years, various approaches have been developed to optimize the liquid biopsy detection method. Especially microfluidic approaches have gained attention, which can be attributed to their high throughput volumes, high sensitivity and specificity, low sampling volumes and lastly their allowances for precise control of the experiments. In this review microfluidic approaches to CTC detection and isolation will be discussed. In addition, the accompanying challenges that are linked to use of microfluidic isolation of CTCs , such as cell heterogeneity, CTC rarity and specificity issues, will be emphasized. Nonetheless, CTCs have great future potential to be exploited as relevant biomarkers for cancer prognosis and disease monitoring. Combining various CTC isolation and detection methods onto single microfluidic chips poses a promising solution to effectively overcome the currently encountered limitations.

Introduction

Cancer is a high-burden disease and the second leading cause of death worldwide. It is estimated that cancer is responsible for a mere 9.6 million deaths in 2018 alone, which translates to 1 in 6 deaths being caused by some form of the disease.[1] The associated mortality can be reduced significantly by means of early detection, comprising both early diagnosis and preliminary screening, as well as by introducing cutting-edge treatment methodologies with a focus on personalised medicine. At the moment, tissue biopsy remains to be the gold standard of cancer diagnosis and subsequent treatment determination. There are, however, some important drawbacks with regard to biopsy, including the invasive nature, complicated tumour accessibility and unaddressed tumour heterogeneity.[2] Consequently, research has been focussed on identifying other approaches to detect and characterize both tumour genetics and dynamics during disease progression. To this end, 'liquid biopsy' has come to the fore over recent years, as a minimally invasive method to analyse cancer-derived materials that have been captured in the bloodstream[3]. A liquid biopsy is defined as a test that is performed on a blood sample in order to detect selected biomarkers, which could be anything ranging from a certain specific protein to excreted cellular waste products.[4] Next to the reduced invasive nature of this approach, another noteworthy advantage is that liquid biopsy can be executed multiple times over the course of tumour progression, thus allowing for the identification of changes of tumour characteristics over time.

As we move into the era of personalized cancer treatment, the development of a more sophisticated and detailed list of biomarkers is needed, which should allow for detection based on their universal instead of individual characteristics.[5]
In light of this, circulating tumour cells (CTCs) have appeared as promising biomarkers to exploit in liquid biopsy, thereby opening up new possibilities for the detection and diagnosis of early stages of de novo or recurrent cancer. CTCs are tumour cells that have acquired an extraordinary mobility that allows them to enter the circulatory system and consequently exploit this system in order to migrate to distant parts of the body. After migration, they can invade and colonize other tissues. These cells have been shown to be largely responsible for the metastatic potential of most cancer types, thereby making them important risk factors for lethality observed among patients.[6,7] Clinical results have pointed to CTCs as good indicators of cancer prognosis, as there is an established relation between the number of CTCs in the peripheral blood and the spread of carcinomas.[8-10] On top of that, it has been shown that enumeration of the CTC concentration in the blood can be used as a pointer to directly predict efficacy of anti-cancer therapy.[11]
Conventional methods for the isolation of biomarkers, such as CTCs, from blood samples have limitations regarding the time, complexity and high costs. On top of that, relatively large volumes of blood are required for the successful isolation of biomarkers, which can only proceed after a time-consuming step of

pre-treatment. Taken together, these disadvantages greatly limit the extent to which genomic analyses can be performed in order to analyse cancer mutations and monitor therapies.[5] Therefore, new approaches have been developed and in recent years great advances have been made regarding the application of microfluidic technologies. In order to create successful CTC detection methods, all the steps that are involved in CTC capture should be carefully considered. These steps include the following: capture from the blood, enrichment of the cells, specific detection and finally release in order to make a (single-cell) analysis.[12] Microfluidic chips allow for the integration of various of these steps onto a single device, creating the opportunity to do everything, from capture to analysis, in a single experimental step. This reduces the loss of rare CTCs by omitting the need to change tubes or filter tips during the experiment. In addition, it makes microfluidics rather cost and time efficient. The advantages that microfluidic strategies provide, has led researchers to develop a range of new microfluidic system-based CTC-detection methods that exploit the unique properties of circulating tumour cells. Such properties include the size, deformability, membrane potential, biomarkers and density of CTCs, which have all been extensively investigated and are characteristics that distinguish CTCs from other haematological cells. In this paper, various CTCs detection methods based on microfluidics will be reviewed, thereby highlighting the emerging role of these devices and CTC detection in developing next-generation point of care (POC) diagnostics.[13] This type of POC diagnostic testing contributes to providing result in a timely manner at the site of the patient, and thus allows for rapid treatment to the patient.[14]

Review

Separation and isolation of CTCs using microfluidic techniques

Microfluidics have gained a large interest in the world of research, mostly due their unique advantageous properties that allow for manipulation of experimental environments as well as due their ability to integrate various experimental approaches on just a single chip. Especially, the development of microfluidic technologies for the detection and isolation of CTCs has seen unprecedented advances, mostly due the increasing interest in cancer diagnostics. Microfluidic approaches can be classified into two broad classes, based on the method of CTC enrichment that is used: label-based and label free methods.[15] The former can be subdivided again in both positive and negative enrichment. Each of these approaches will be discussed in more detail, substantiated with the most important technical examples that have been described in recent publications (see table 1).

Immune-affinity based detection techniques (label-based)

Labelled isolation and detection methods are based on the use of biomarkers or molecular recognizers that are cell type specific. Examples of such molecular recognizers are antibodies, sialic acid and peptides.[16] Extensive research on characterizing CTCs has provided a deep understanding and extensive list of various CTC biomarkers. The advantage of this method is the high specificity that can be reached regarding isolation of one specific cell type. Nevertheless in the context of detecting CTCs, this ensures that heterogeneity of tumour cells (which have varying surface protein expression profiles) cannot be addressed, which is a big disadvantage.

Positive enrichment of CTCs

Positive enrichment methodologies refer to approaches that are based on the specific separation of a target cell using the characteristics of that particular cell type, such as cell-surface protein expression and size. In the case of CTCs, positive enrichment thus encompasses the isolation of CTCs from the peripheral bloodstream. Epithelial cell adhesion molecule (EpCAM) is mainly used as the specific biomarker among positive cell sorting assays. This molecule is involved in the adhesion between epithelial cells as well as in signal transduction pathways, and has been shown to be frequently upregulated in primary and metastatic cancer.[17] Considering that CTCs have an epithelial origin, they were hypothesized to have a relatively high-affinity for EpCAM antibodies. In 2004, the first positive enrichment

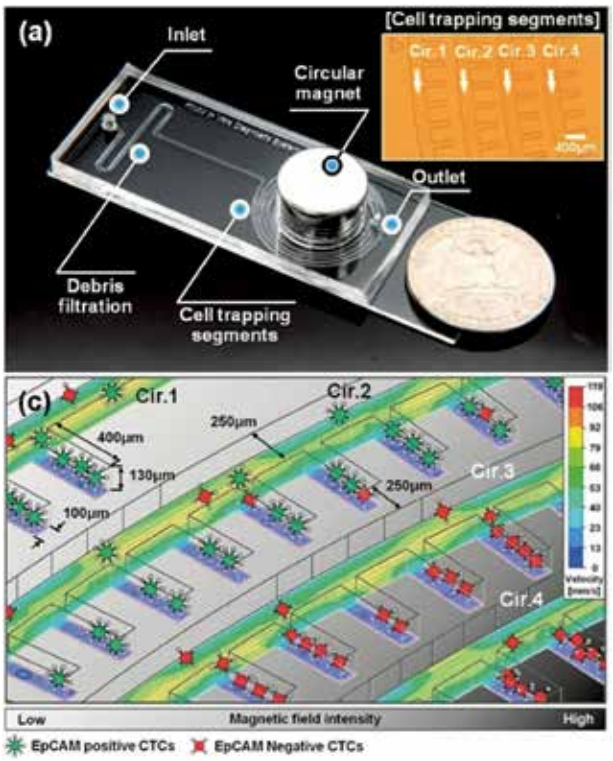
Table 1 - Overview microfluidic technologies for the detection and isolation of CTCs

Platform	Enrichment principle	Pros	Cons	
	Label-based			
CellSearch	Positive enrichment (EpCAM)	FDA approved, clear guidelines	CTC heterogeneity unaddressed, rarity of CTCs, low yield and specificity	(40)
Herringbone Chip	Positive enrichment (EpCAM)	Increased surface area for cell capture	CTC heterogeneity unaddressed	(19)
Micropost CTC-Chip	Positive enrichment (EpCAM)	Increased specificity and yield	CTC heterogeneity unaddressed	(18)
Spiral shaped microchannel	Positive enrichment (EpCAM)	Addresses EpCAM heterogeneity, high efficiency	Specificity remains issue	(20)
Dual-patterned immunofiltration (DIF)	Negative enrichment (CD45)	no direct interaction with CTCs, no need for purification	Low throughput volume	(25)
Two-stage microfluidic chip	Negative enrichment (CD45) and positive enrichment (EpCAM, HER2)	High throughput	Clogging of channels	(24)
	Label-Free			
3D microfilter device	Size	High viability	Non-specific, clogging, unaddressed cell-deformability	(27)
Size-based microfluidic chip	Size (triangular pillar arrays)	Reduced cell clustering, efficient use	Limited yield, clogging	(28)
Dielectrophoretic (DEP) device	Variable dielectric properties Acoustophoretic technology	High specificity (approaching immune affinity based specificity)		(34)
Acoustic cell separation		Preserves cell integrity (phenotype/ genotype)		(35)

method for CTCs was approved by the Food and Drug Administration (FDA), which was the CellSearch platform. This is a microfluidic system in which EpCAM antibodies are conjugated with magnetic nanoparticles. When a blood sample is injected into the device and subsequently flows through the microfluidic channels, CTCs (with a much higher affinity for EpCAM than leukocytes), bind to anti-EpCAM. Subsequently, they are immobilized to the surface of the channels with the help of a magnetic field. On the other hand, the remaining blood, that is not bound by the antibodies is driven away from the microfluidic channels and leaves the device. Afterwards enumeration steps can be performed, by means of staining with fluorescent markers and imaging technologies.[12] With the advent of this platform, many more enumeration and detection technologies based on EpCAM have been generated. Most of these positive selection methods achieve CTC enrichment by either coating antibodies to the surfaces of microfluidic channels or by conjugating the antibodies to specific micro particles/structures on the microfluidic chips. With regard to this approach, the limitation that has to be overcome is that there is a limited capture capacity due the relatively small surface area of the ligating antibodies. Microfluidic chips have been designed that have an increased surface area on which the interactions between antibody and target cell can take place, thereby increasing the number of such interactions. For example the CTC-chip that uses microposts and the herringbone-chip that integrates a microfluidic mixing device, have both shown increases in the rate of affinity between (EpCAM) antibodies and CTCs, resulting in more efficient isolation.[18,19] Another microfluidic channel that was recently proposed by Kwak et al. was spiral shaped, as shown in figure 1.[20] This geometry allowed for the capture of CTCs with varying levels of EpCAM expression. EpCAM antibodies were conjugated with magnetic nanoparticles such that after recognition each CTC would have a certain magnetic charge based on its expression level. Next, the detected CTCs could be separated by applying a magnetic field gradient. The spiral contains a fixed number of CTC binding sites and can therefore selectively position the recognized cancer cells.

Besides epithelial markers such as EpCAM, there is also a range of detection methods that is based on markers that are more cancer cell specific.[11] This list is extensive and still expanding, and includes markers such as epidermal growth factor receptor (EGFR), epidermal growth factor receptor 2 (HER2), which is an important marker for breast cancer, prostate-specific antigen (PSA), among many others.[21] As becomes evident, none of these markers are universally expressed in every type of cancer, not even in all cells of a particular tumour type. This heterogeneity of tumours is one of the main hurdles that is faced by immune-affinity based detection methods and contributes to the underestimation of CTCs in the bloodstream.[11] This limitation is further aided by the fact that contamination of the isolated CTCs can arise, due to leukocytes that have acquired positive expression of the same markers that the CTCs have an affinity for, thereby introducing false-positive detection of cells. If we take the biomarker EpCAM as an example, it has been acknowledged that the expression of this surface protein in CTCs changes over the course of cancer progression. EMT (epithelial to mesenchymal transition) is known to play a major role in

Figure 1 - Spiral shaped microfluidic channel with distinct CTC binding sites depending on magnetic charge. Extracted from: [20]

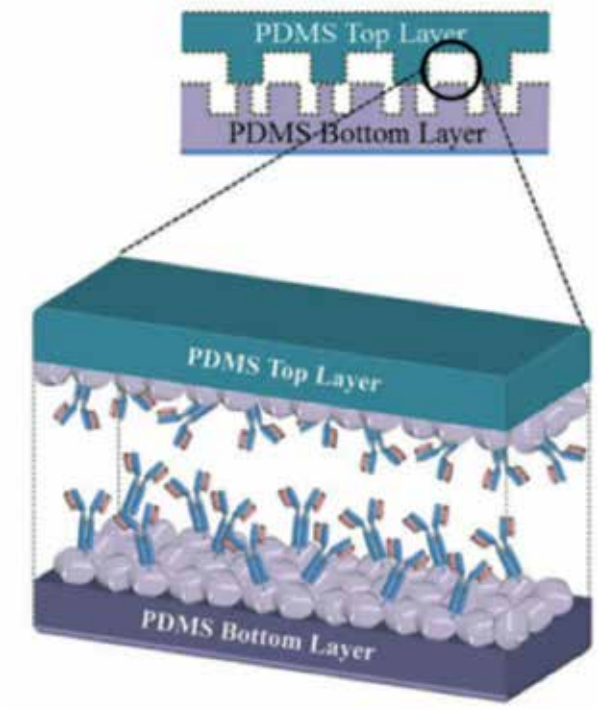


cancer metastasis.[22] This process causes the downregulation of epithelial cell markers, such as EpCAM, and thereby gives rise to CTCs that have an increased mobility and potential for metastasizing. These are the type of circulating cancer cells that may actually present themselves as the most important source of recurrent or aggressive cancers. The direct result of the EMT process is that, EpCAM may not be such a good marker for cancer diagnosis and prognosis after all. CTCs that have undergone the transition to more mesenchymal like cells, are missed in the microfluidic detection methods based on EpCAM conjugation. This recognition has led to the hypothesis that label-free methods or negative enrichment for CTC detection may harness higher capture efficiency compared to labelled approaches.

Negative enrichment of CTCs

As stated before, tumours are complex heterogeneous tissue types that contain multiple contributing cell types rather than one individual clone.[23] Rationally, this means that the concentration of surface antigens varies within the different tumour cell lines. As a result positive enrichment methods, that make use of specific surface protein expression profiles, are limited in their applicability. As an alternative, negative enrichment methods have been developed, in which leukocytes are considered to be the target cells rather than the circulating tumour cells themselves. This implies that CTCs with heterogenic properties are isolated through specific elimination of blood cells.[24]The most common approach is a microfluidic channel that contains CD45 immobilized antibodies on the surface of its channels. Bu et. Al. [25] developed a dual-patterned immunofiltration (DIF) device (figure 2), which aligns specifically designed top and bottom layers that have been functionalized with anti-CD45.This

Figure 2 - Dual-patterned chips as developed by Bu et al. for the negative enrichment of CTCs. Extracted from: [25]



geometry significantly increases the binding chances of the common surface antigens found on leukocytes to the created immune pattern on the device, allowing for rapid negative selection. An additional advantage is, that the CTCs are not touched by antibodies or other interacting structures, which eliminates the need for purification and release steps in order to obtain isolated CTCs.[25] The performance of the device was tested with a human non-small lung (NSCL) cancer cell line and this reported an elimination of even more than 97% of leukocytes at a flow rate of 1mL/h. However, it must be noted here that the throughput remains rather low, making this a time consuming technology.

To address the throughput issue Hyun et. al. developed a two-stage microfluidic chip, which consist of two consecutive different types of microfluidic chips.[24] The first one can be characterized as a microfluidic magnetic-activated cell sorter (μ-MACS), which can extract magnetically labelled cells from the input sample. In this case, white blood cells coated with CD45 antibody-conjugated magnetic nano-particles were eliminated at this stage of the process. CTCs had no magnetic charge, and these heterogeneous cells were thus free to flow onto the next microfluidic chip. This second part consists of a geometrically activated surface interaction (GASI) chip, which functions based on interactions between the microfluidic channel surfaces and target cells. The GASI chip ensures successful capture of CTCs, depending on the antigen-antibody interactions (EpCAM and HER2). This latter part of the method can be considered as positive enrichment again. Even though, this method proposed by Hyun et al.[24] significantly increased the throughput, the problem of clogging of the microfluidic channels remains. Considering that CTCs are a rare cell type in the blood, large volumes of sample have to be separated in order to obtain a sufficient amount of CTCs. When all of the white blood cells are collected in the channels by means of magnetic forces, these will run out of space rather quickly. This clogging problem continues to be an issue of concern.[17]

Label free CTC detection techniques

This group of microfluidic technologies is foremost based on physical cellular properties, such as size, density and deformability and thereby circumvents the problem of heterogeneous cell surface markers in circulating cancer cells.

Separation based on size

CTCs mainly originate from epithelial cells, which contributes to the morphological differences that can be observed when compared to normal blood cells. CTCs are larger in size than blood cells, and therefore size-based microfluidic devices have been developed.[26] Zheng et al.[27] designed a 3D microfilter device, that consists of two layers of parylene membrane containing pores and gaps of specific dimensions. These micro sieves can be fabricated with precisely defined photolithography, making sure that the smaller blood cells can pass unaltered and that the CTCs are captured by the membranes.[27] Another, more sophisticated, size-based microfluidic chip as shown in figure 2 was proposed by Gao et al..[28] This chip contains main channels (the bulk filtration area), as well as smaller side channels (the filtration channels). CTCs are contained in the main channels as their size surpasses the size of the openings in the single cell filtration membranes. On the other hand the red and white blood cells successfully pass into the side filtration arrays, thereby purifying the CTCs in the main channel. The efficiency of this device is high and allows for easy processing of blood samples. However there is still a problem of clogging as the side channels will fill up with accumulated blood cells. In addition, concerns are expressed about overlapping sizes of leukocytes and smaller CTCs. This poses limits on the isolation yield, as the smaller CTCs can still pass the filtration membranes and are therefore lost during the process.[29]

Another noteworthy development related to size separation, is the finding that CTC clusters have a 23 to 50-fold increased potential for metastasis compared to single CTCs.[30] CTC clusters are multicellular groupings of primary tumour cells and acquire their higher metastatic potential due to an increased cell-cell adhesion between single CTCs combined with the fact that such clusters can interact and combine with surrounding blood cells. Both of these observations contribute to efficient escape from immune surveillance attacks. Considering the significance of CTC clusters in relation to cancer prognosis, methods have been proposed to isolate these clusters rather than individual cells. Clusters are particularly relevant, because they decrease the size overlap between leukocytes and the isolation targets.

Separation based on dielectric properties

Another label free method of detecting CTCs uses the differences in dielectric properties between CTCs and other circulating cells.[31] Namely, the capacitances of malignant CTCs differ from normal cells. The advantage of this method is that dielectrophoresis (DEP) can almost reach the same specificity of immune-affinity based strategies, which is not shared with

other label free technologies. It can isolate and identify cells with distinct phenotypes, which suits very well for the detection of CTCs. The general idea of DEP is that cells get polarized once they enter an electric field gradient. Because this electric field does not have the same strength at each spatial position, charges get non-uniformly distributed over cell membranes, thereby exerting a net force on the cell and thus providing it with directionality. The microfluidic environment, ensures that a cell is subject to a combination of forces, which ultimately results in each different cell population carrying a specific charge depending on the cell's properties. Because cancer cells have been shown to have unique characteristics and dielectric properties, including protein concentration, protein glycosylation and increased membrane roughness, DEP can be used to isolate CTCs from the blood.[32,33] Considering that CTCs have a higher polarisation potential compared to other cells in the blood, they will migrate to the periphery of the electrode side, resulting in efficient separation.

One particularly interesting approach that exploits the DEP technique, was reported by Cheng et al..[34] In this three dimensional microfluidic device (figure 4), an electric field gradient along a V-shaped microchannel induces a lateral dielectrophoretic force normal to the flow direction of the blood cells. This allows for the manipulation of CTCs and blood cells, which causes them to come to their specific equilibrium positions in the channel. Considering the differences in dielectric properties, sizes and shapes, the molecules can effectively be separated in different downstream subchannels.

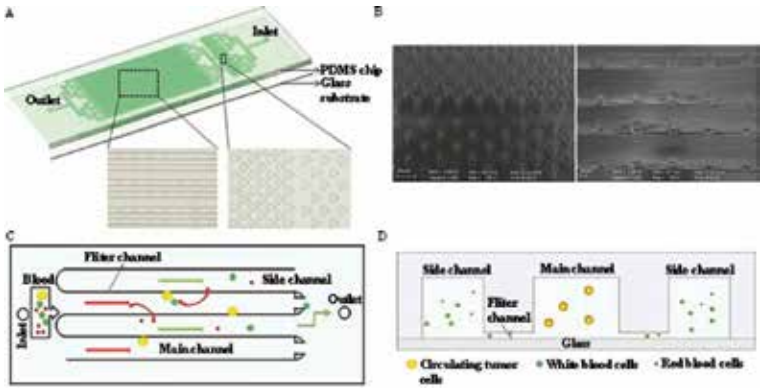
Separation based on acoustophoretic techniques

Many CTCs separation methods interfere with the biology of the target cells in some way or another, which poses limitations on the successful and intact final CTCs isolation. In order to avoid this, Peng Li et al.[35] produced an acoustic cell separation device that exploits the differences in size, compressibility and other physical properties of cells.[25] Methods based on acoustics are known to preserve integrity, functionality and viability of target cells, and therefore have an advantage over other conventional separation methods. This acoustic-based microfluidic device makes use of tilted-angle standing surface acoustic waves that exert an acoustic radiation force on the cells that pass the microfluidic channel. CTCs are larger in size than white blood cells, and as a result experience larger such forces. Therefore, they show a bigger vertical displacement, allowing for capture of the CTCs in separate channels.

Integrated microfluidic approaches

While the described methods have shown favourable performances, all of them are still subject to a number of important drawbacks. Immuno-affinity based detection methods are unable to address the heterogeneity that is found in CTCs, even though this is an important characteristic of cells that have metastatic potential. On the other hand, label free approaches have an higher efficiency and can capture CTCs that have varying affinity binding properties. Yet, this comes at the cost of specificity and therefore results in impurities in the isolated cells. Taking these hurdles into account, researchers have focussed attention on integrating both methods in a single chip.[15] One example

Figure 3 - Design and operation of size based microfluidic chip. Cell types and channels are indicated. Extracted from [28]

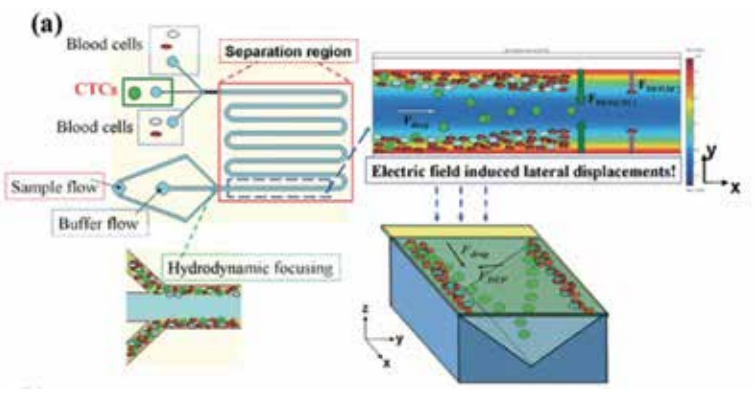


that has shown good results in terms of detection and isolation efficacy, is the CTC-iChip platform, which combines three different microfluidic components.[36] Firstly, deterministic lateral displacement (DLD) gradually separates nucleated cells from red blood cells and platelets. This sorting is achieved due to the differences in lateral displacement as a result of differences in laminar flow characteristics of the differentially shaped cells.

Next, the remaining cells pass an inertial focusing step that enriches the cells of interest. Finally, conjugation with magnetic nanoparticles finalizes the separation process and results in a precise accumulation of CTCs in predefined wells. More and more integrated microfluidic designs are coming to the surface. This provides new opportunities to optimize the isolation and detection of CTCs, as the limitations of each individual method can be lightened. In addition, integrative technology on microfluidic chips reduces the required experimental steps, decreases the needed sample volume and simultaneously facilitates analyses of the isolated product in a single step.

Thus far, the role of microfluidics in CTC separation and detection steps have been discussed. However, subsequent quantitative analysis of CTCs requires both cell enumeration and reversibility of conjugation reactions. This forms another challenge that will have to be addressed in more depth. After isolation, CTCs should remain viable and pure, as well as detached from the microfluidic surfaces and antibodies. Only then is subsequent phenotypic and genotypic cellular characterization possible. This characterization is necessary for future therapy

Figure 4 - Lateral displacement DEP CTCs separation technique. Induced forces bring cells with varying properties to their distinct equilibrium position. Extracted from [34]



guidance, as you need full information about the isolated cells before any treatment can be administered. Various microfluidic approaches have incorporated single CTC analysis methods and next generation sequencing approaches directly onto the same platform used for isolation. This results in immediate characterization of CTCs in a single step. Such an example is the microfluidic device developed by Chen et al.[37] which exploits field-effect transistors for the enumeration of cells. Successful, non-destructive cell release was achieved by Yu. et all.[38], by means of the integration of air foam technology that induces a high shear stress and thereby releases captured cells from the microfluidic surfaces. This resulted in a comparatively high cell-recovery rate.

Another research topic related to single-cell analysis is identification of cells that are responsible for metastasis. Since circulating tumour cells can give rise to new cancerous colonies, metastatic potential can be characterized by profiling individual CTCs.

Lately, the cancer stem cell model has gained significant support from researchers and it would be interesting to link cancer stem cell research to developments that are being made in CTC profiling, as these two separate topics might well be correlated with each other. Another future implication would be to combine CTC isolation technologies with the production of chimeric antigen receptor modified T-cells (CAR-T) therapies.[39] In CAR-T therapy, the patient derived T-cells are functionalized to cause a specific immune response against cancer cells. By isolating CTC cells (known for their association with higher metastatic potential) and characterizing them, this information could be used to create CAR-T therapies targeted at cancer stem cells. The ultimate goal would be to design microfluidic chips, such that isolation of CTCs and production of CAR-T cells can all take place on the same integrated device, thereby making it an highly-efficient approach.

Discussion

To date, enormous advances have been made in the field of CTC detection and isolation based on microfluidic approaches. These methods can be divided into immune-affinity based approaches (involving both positive and negative enrichment), and label free methods. Neither one of those microfluidic platforms can be said to be superior to the other, as each technique remains subject to important limitations. Consequently, not many devices have been approved by the FDA for clinical use. Important hurdles still need to be overcome before the fully potential of CTC research can be realized and translated to clinical settings. One of the major challenges in CTC isolation and detection, remains the intrinsic rarity and heterogeneity of CTCs, making it hard to reach sufficient numbers of isolated CTCs for analysis. Microfluidic approaches must therefore include enrichment steps and at the same time be very sensitive and specific in order to obtain the highest possible yield. Promising results have been reported by integrating different methods onto single microfluidic devices, thereby maximizing the advantages of the individual methods, yet reducing the disadvantages.

Overall microfluidics contribute to novel detection platforms that are characterized by high throughput, high sensitivity and specificity, smaller sample requirements, lower costs and time-efficiency, which all contribute to successful separation of CTCs. Microfluidic technologies are on the way to transform the field of cancer diagnostics and research, while greatly contributing to our understanding of phenotypic properties of cells with higher metastatic potential.

References

1. *Plummer M, de Martel C, Vignat J et al. Global burden of cancers attributable to infections in 2012: a synthetic analysis. Lancet Glob Heal. 2016; 4:609–616.*
2. *Ilié M, Hofman P. Pros: Can tissue biopsy be replaced by liquid biopsy? Transl lung cancer Res. 2016; 5:420–423.*
3. *Marrugo-Ramírez J, Mir M, Samitier J. Blood-Based Cancer Biomarkers in Liquid Biopsy: A Promising Non-Invasive Alternative to Tissue Biopsy. Int J Mol Sci. 2018; 19.*
4. *Definition of liquid biopsy - NCI Dictionary of Cancer Terms - National Cancer Institute [cited 2019 Mar 7]. Available from: https://www.cancer.gov/publications/dictionaries/cancer-terms/def/liquid-biopsy*
5. *Lewis JM, Heineck DP, Heller MJ. Detecting cancer biomarkers in blood: challenges for new molecular diagnostic and point-of-care tests using cell-free nucleic acids. Expert Rev Mol Diagn. 2015; 15:1187–1200.*
6. *Ito H, Sato J, Tsujino Y, et al. Long-term prognostic impact of circulating tumour cells in gastric cancer patients. World J Gastroenterol. 2016; 22:10232–10241.*
7. *Bailey PC, Martin SS. Insights on CTC Biology and Clinical Impact Emerging from Advances in Capture Technology. Cells. 2019; 8:553.*
8. *Cristofanilli M, Pierga J-Y, Reuben J, et al. The clinical use of circulating tumor cells (CTCs) enumeration for staging of metastatic breast cancer (MBC): International expert consensus paper. Crit Rev Oncol Hematol. 2019; 134:39–45.*
9. *Cristofanilli M, Budd GT, Ellis MJ, et al. Circulating Tumor Cells, Disease Progression, and Survival in Metastatic Breast Cancer. N Engl J Med. 2004; 351:781–791.*
10. *Cohen SJ, Punt CJA, Iannotti N, et al. Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. J Clin Oncol. 2008; 26:3213–3221.*
11. *Sleijfer S, Gratama J-W, Sieuwerts AM, et al. Circulating tumour cell detection on its way to routine diagnostic implementation? Eur J Cancer. 2007; 43:2645–2650.*
12. *Shen Z, Wu A, Chen X. Current detection technologies for circulating tumor cells. Chem Soc Rev. 2017; 46:2038–2056.*
13. *Kulasinghe A, Wu H, Punyadeera C, et al. The Use of Microfluidic Technology for Cancer Applications and Liquid Biopsy. Micromachines. 2018; 9:397.*
14. *Department of Health | Definitions - Point-of-care testing [Internet]. [cited 2019 Oct 3]. Available from: https://www1.health.gov.au/internet/publications/publishing.nsf/Content/qupp-review~qupp-definitions*
15. *Dan Z, Daxiang C. Advances in isolation and detection of circulating tumor cells based on microfluidics. Cancer Biol Med. 2018; 15:335.*
16. *Zhao Y, Xu D, Tan W. Aptamer-functionalized nano/micro-materials for clinical diagnosis: isolation, release and bioanalysis of circulating tumor cells. 188 | Integr Biol. 2017; 9:188.*
17. *Gwak H, Kim J, Kashefi-Kheyrabadi L, et al. Progress in Circulating Tumor Cell Research Using Microfluidic Devices. Micromachines. 2018; 9:353.*
18. *Nagrath S, Sequist L V., Maheswaran S, et al. Isolation of rare circulating tumour cells in cancer patients by microchip technology. Nature. 2007; 450:1235–1239.*

19. *Stott SL, Hsu C-H, Tsukrov DI, et al. Isolation of circulating tumor cells using a microvortex-generating herringbone-chip. Proc Natl Acad Sci. 2010; 107:18392–18397.*
20. *Kwak B, Lee J, Lee J, et al. Spiral shape microfluidic channel for selective isolating of heterogenic circulating tumor cells. Biosens Bioelectron. 2018; 101:311–316.*
21. *Li W, Wang H, Zhao Z, et al. Emerging Nanotechnologies for Liquid Biopsy: The Detection of Circulating Tumor Cells and Extracellular Vesicles. Adv Mater. 2018; 1805344.*
22. *Barriere G, Fici P, Gallerani G, et al. Circulating tumor cells and epithelial, mesenchymal and stemness markers: characterization of cell subpopulations. Ann Transl Med. 2014; 2:109.*
23. *de Bruin EC, McGranahan N, Mitter R, et al. Spatial and temporal diversity in genomic instability processes defines lung cancer evolution. Science. 2014; 346:251–256.*
24. *Hyun K-A, Lee TY, Lee SH, et al.Two-stage microfluidic chip for selective isolation of circulating tumor cells (CTCs). Biosens Bioelectron. 2015; 67:86–92.*
25. *Bu J, Kang Y-T, Kim YJ, et al. Dual-patterned immunofiltration (DIF) device for the rapid efficient negative selection of heterogeneous circulating tumor cells. Lab Chip. 2016; 16:4759–4769.*
26. *Lee TY, Hyun K-A, Kim S-I, et al. An integrated microfluidic chip for one-step isolation of circulating tumor cells. Sensors Actuators B Chem. 2017 J; 238:1144–1150.*
27. *Zheng S, Lin HK, Lu B, et al. 3D microfilter device for viable circulating tumor cell (CTC) enrichment from blood. Biomed Microdevices. 2011; 13:203–213.*
28. *Gao W, Yuan H, Jing F, et al. Analysis of circulating tumor cells from lung cancer patients with multiple biomarkers using high-performance size-based microfluidic chip. Oncotarget. 2017; 8:12917–12928.*
29. *Micalizzi DS, Maheswaran S, Haber DA. A conduit to metastasis: circulating tumor cell biology. Genes Dev. 2017; 31:1827–1840.*
30. *Fabisiewicz A, Grzybowska E. CTC clusters in cancer progression and metastasis. Med Oncol. 2017; 34:12.*
31. *S. Iliescu F, Sim WJ, Heidari H, et al. Highlighting the uniqueness in dielectrophoretic enrichment of circulating tumor cells. Electrophoresis. 2019; 40:1457-1477*
32. *Nourse JL, Prieto JL, Dickson AR, et al. Membrane biophysics define neuron and astrocyte progenitors in the neural lineage. Stem Cells. 2014; 32:706–716.*
33. *Gascoyne PRC, Shim S, Noshari J, et al. Correlations between the dielectric properties and exterior morphology of cells revealed by dielectrophoretic field-flow fractionation. Electrophoresis. 2013; 34:1042–1050.*
34. *Cheng I-F, Huang W-L, Chen T-Y, Liu C-W, Lin Y-D, Su W-C. Antibody-free isolation of rare cancer cells from blood based on 3D lateral dielectrophoresis. Lab Chip. 2015; 15:2950–2959.*
35. *Li P, Mao Z, Peng Z, et al. Acoustic separation of circulating tumor cells. Proc Natl Acad Sci U S A. 2015; 112:4970–4975.*
36. *Karabacak NM, Spuhler PS, Fachin F, et al. Microfluidic, marker-free isolation of circulating tumor cells from blood samples. Nat Protoc. 2014; 9:694–710.*
37. *Chen Y-H, Pulikkathodi AK, Ma Y-D, et al. A microfluidic platform integrated with field-effect transistors for enumeration of circulating tumor cells. Lab Chip. 2019; 19:618–625.*
38. *Yu C-C, Chen Y-W, Yeh P-Y, et al. Random and aligned electrospun PLGA nanofibers embedded in microfluidic chips for cancer cell isolation and integration with air foam technology for cell release. J Nanobiotechnology. 2019; 17:31.*
39. *Guo Y, Feng K, Wang Y, et al. Targeting cancer stem cells by using chimeric antigen receptor-modified T cells: a potential and curable approach for cancer treatment. Protein Cell. 2018; 9:516–526.*
40. *CELLSEARCH® | Home [Internet]. [cited 2019 Oct 3]. Available from: https://www.cellsearchctc.com/*

Systematic Review

A Meta-analysis of the One-Year Survival after Combined Lung-Liver Transplantation

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Abstract

***Background*: Combined lung liver transplantation (LLTx) can be performed in patients with cystic fibrosis, hepatopulmonary fibrosis, portopulmonary hypertension and cirrhosis, who are suffering from end-stage lung and liver disease. We investigated outcomes from combined LLTx and surgical approach of LLTx.**

***Methods:* PubMed was searched for articles published until December 2018 on results of combined LLTx. We primarily analyzed one-year survival and assessed whether this was affected by the sequence of organ transplantation. We compared the one-year survival of the liver first principle, with the one-year survival of the lung first principle. Secondarily, we assessed which factors were associated with survival.**

***Results:* The search strategy yielded 178 articles, of which 10 articles were included in the analysis. The one-year survival after combined LLTx was 75,2%. The one-year survival of LLTx with liver implantation first is 100,0% and with lung implantation first 90,9%.**

***Conclusion:* Combined LLTx has a slightly lower one-year survival rate compared to single lung/liver transplantation, respectively 75,2% and 80,0%. No clear statement can be made regarding the effects of implantation order on the one-year survival.**

Introduction

The first combined lung liver transplantation (LLTx) was performed in 1994. [1] Since then 122 patients have been waitlisted in the United States until 2010. With 43,4% of those waitlisted in the last four years. [1] To this day, 114 patients have received a combined LLTx in the USA. [2] Since 1988 in the USA there have been performed over 450 thousand kidney transplants, 73 thousand heart transplants and 165 thousand liver transplants. [3] These numbers indicate just how rare the combined LLTx is.
The most common indications for LLTx are cystic fibrosis, hepatopulmonary fibrosis, portopulmonary hypertension, cirrhosis, hepatic steatosis, α1 antitrypsin deficiency and hepatitis C. [4,5] Patients with both end stage liver and lung disease, are considered qualified for a combined LLTx when no other treatment is applicable. [6]
The lungs are less able to withstand a long ischemic time than the liver. [6] The lungs can withstand a ischemic time of six hours, compared to twelve hours for the liver. [7] Therefore, combined LLTx is usually performed with implantation of the lungs first.
However, with the introduction of ex vivo lung perfusion, the method of the liver first principle was developed. This means that with the new method of ex vivo lung perfusion, the liver could be transplanted before the lungs, hence the name; the liver first principle. [6] Some studies argue this liver first approach reduces exposure of complications to the newly implanted lungs such as coagulopathy, significant transfusion of blood products, blood loss and hemodynamic instability which regularly occurs during liver transplantation surgery. [6] It remains unknown if this is a better option in all patients.
Ceulemans et al. described a transplantation sequence based on which organ has the worst function and transplant this organ first. [4]
Because of illnesses like Cystic Fibrosis, patients are more often in need of transplantation of multiple organs, like lungs and liver. There have been performed combined LLTx in patients with CF and end stage liver- and lung disease. Researching outcomes of combined transplantation in one surgery or multiple transplantations in multiple surgeries could lead to optimization of patient care and survival.
With increasing needs for transplantation, it is clinically important that combined lung liver transplantation and the survival rates are investigated. More data will lead to more hospitals being able to perform combined LLTx for patients suffering from end-stage lung and liver disease.
The goal of our study is to research the one-year survival and the effects of the surgery technique on the one-year survival. Furthermore, we also researched the influence of patient charac-

teristics such as recipient BMI and recipient age and their effect on the one-year survival.

Methods

Search

The search was conducted in December 2018 in the PubMed database using the following (Major) terms: "Liver-Lung transplantation" OR "Lung-Liver Transplantation" OR ("Lung Transplantation"[Majr] AND "Liver Transplantation"[Majr]).

The reason of the use of major terms is that we aimed to find articles that have combined LLTx as main objective of their research.

Three reviewers independently screened studies based on title and abstract for possible inclusion when available in full text at our institution. Articles that finally did not have combined LLTx as main topic were excluded. The three reviewers then independently read the articles completely, from which the final articles for the review were selected.

Inclusion criteria were patient and/or graft survival had to be mentioned, available at Erasmus MC, studies published in English, patient population underwent LLTx. Exclusion criteria were: only describing pediatric patients, patient populations without combined LLTx or case reports.

Our primary outcome is the proportion of one-year patient survival after combined LLTx. Our secondarily outcomes are the effect of lung transplantation first, liver transplantation first, recipient BMI and recipient age on the one-year survival.

Quality assessment

Quality assessment was conducted by two independent reviewers. We used a scale we designed ourselves, the Rotterdam Medical Students Scale (RMSS). The criteria of the Rotterdam Medical Students Scale that is based on the Newcastle Ottawa Scale was used [8] which includes parameters such as mentioning of patient characteristics, length of follow up, mentioning of inclusion and/or exclusion criteria and number of patients included in the studies. See appendix 1 for the criteria of the RMSS.

If there was an inconsistency in the quality assessments, the assessors tried to find consensus and if this was not reached a third reviewer was requested to draw a final conclusion.

We quantified articles with high quality when a score of 3 or 4 was given. Articles with a quality score of 0, 1 or 2 were qualified as low quality studies. Therefore, the maximum score of the RMSS is 4 points and the minimum score is 0 points.

Statistical analysis

One-year survival data was deducted from the percentages from the studies.

The meta-analysis regarding the proportion of the one-year survival was conducted with the program OpenMeta[analyst] version of 11-10-2012. The p-value for heterogeneity was set at 0,1. Any value greater than 0,1 would mean the studies are homogeneous and therefore the fixed effects analysis would be conducted instead of the random analysis.

We performed a meta-analysis with all the included studies and a sensitivity analysis, only including the studies with high quality (3 points or higher).

Secondarily, we stratified the meta-analysis for transplantation sequence, liver first, lungs first or unknown. The reason for comparison of the different methods of implantation is to research if there is an optimal method.

Table 1 - Overview of the included studies and study characteristics

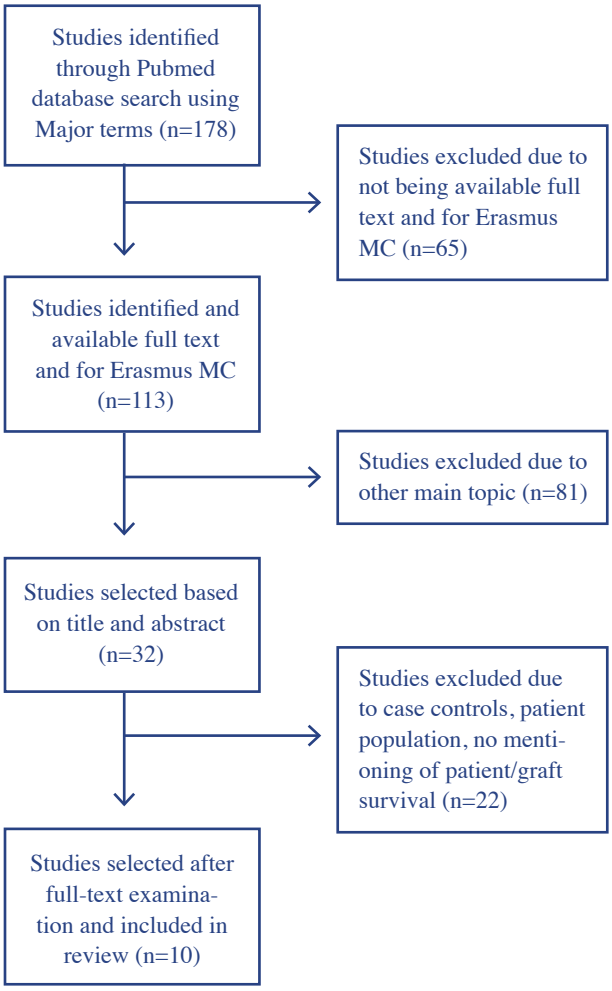
Title	Authors	Publishing year	Study type	Number of LLTx patients	One year survival	Operation type	Mean recipient BMI (kg/m2)	Mean recipient age (years)
Combined lung and liver transplantation in patients with cystic fibrosis. A 4 1/2-year experience.	Couetil et al. [12]	1995	Retrospective cohort study	5	60,0-70,0%	Lung first	16,7	-
Combined lung and liver transplantation: The United States experience.	Barshes et al. [13]	2005	Case report / meta analyse /review	11	79,0%	Unknown	17,2	15,0
Indications for and outcomes after combined lung and liver transplantation: a single-center experience on 13 consecutive cases.	Grannas et al. [14]	2008	Retrospective cohort study	13	69,0%	Lung first	20,8	35,0
Liver and combined lung and liver transplantation for cystic fibrosis: analysis of the UNOS database.	Arnon et al. [11]	2011	Review	15	80,0%	Unknown	-	20,1
Survival of cystic fibrosis patients undergoing liver and liver-lung transplantations.	Desai et al. [10]	2013	Retrospective cohort study	29	83,0%	Unknown	-	-
Simultaneous thoracic and abdominal transplantation: can we justify two organs for one recipient?	Wolf et al. [1]	2013	Retrospective cohort study	42	75,5%	Unknown	21,3	32,3
Combined lung and liver transplantation: analysis of a single-center experience.	Yi et al. [15]	2014	Retrospective cohort study	6	71,4%	Lung first	19,1	42,5
Combined liver-thoracic transplantation: single-center experience with introduction of the 'Liver-first' principle.	Ceulemans et al. [4]	2016	Retrospective cohort study	10	100,0%	7 Lung first 3 Liver first	-	42,0
Conquering combined thoracic organ and liver transplantation: indications and outcomes for heart-liver and lung-liver transplantation.	Yi et al. [5]	2018	Review	14	85,7%	Unknown	21,8	58,8
Single-Center Long-Term Analysis of Combined Liver-Lung Transplant Outcomes	Freischlag et al. [16]	2018	Retrospective cohort study	12	90,0% Lung first 100,0% Liver first 91.7% total	11 Lung first 1 Liver first	20,7	28,4

With the help of the CI Calculator, we calculated the difference in one-year survival between the meta-analysis including all the studies, and the analysis including only the high quality studies. [9]
To determine whether there was a relation between the one-year survival, recipient age and recipient BMI, we calculated the square Pearson Correlation Coefficient, R2, in Excel version 1811. A clear correlation is found when R2 is 0,95 or greater.

Results

Search results
With our search strategy using the major terms, we identified 178 articles of which 113 articles were available full text or at our institution. With 3 reviewers, we independently selected 32 articles based on title and abstract. After full-text analysis, we identified 10 articles which fulfilled all criteria and were therefore used for this meta-analysis (flowchart figure 1).
An overview of the 10 articles selected for our review can be found in table 1.

Figure 1- Flowchart of article selection based on our inclusion and exclusion criteria.



With our selection of studies, we collected data of a total of 151 patients who underwent combined liver and lung transplantation. In some articles, the data of these patients was compared to patients who underwent a liver transplantation (LTx, n = 262.808). [4,10,11]

Quality assessment
7 out of 10 articles received a quality score of 3. [1,11-16] 2 articles received a score of 2. [4,10] And 1 article received a score of 1. [5] Appendix 2 shows the scores the articles received based on the quality assessment using the Rotterdam Medical Students Quality Scale.
We again conducted the meta-analysis, only including those articles with a high quality score of 3 or 4. The results of the 7 articles with high quality, that are included in the meta-analysis are shown in figure 2B. With an I2 value of 8,0%, we found little heterogeneity between these articles. This meta-analysis shows that of the 106 patients included in the high quality analysis, 77 of them were alive one year after transplantation. The one-year survival in our analysis is 73,1%.
The difference between the meta-analysis, including all studies, and the meta-analysis, including only the high quality studies is 0,0214 (95% CI -0,0845; 0,1319,).

Primary endpoint
Mean recipient age of the 151 patients included is between 15 and 58,8 years. Most people underwent combined LLTx because of organ failure caused by cystic fibrosis, hepatopulmonary fibrosis, portopulmonary hypertension, cirrhosis, hepatic steatosis, α1 antitrypsin deficiency and hepatitis C. [4,5]
The results of the meta-analysis on the proportion of the one-year survival are shown in figure 2A.
We found little heterogeneity regarding the one-year survival within the included studies, I2 = 35,0%.
Of the 151 patients included in the meta-analysis, 114 of them were alive after one year. The one-year survival is 75,2%.

Secondarily endpoints/sensitivity analysis
Five articles described patients who underwent combined LLTx where the lung was transplanted before the liver. [4,12,14-16] These results can also be found in table 1. We found little heterogeneity regarding the difference in one-year survival between the different studies who transplanted the lung first. I2 = 7,0%. Of the 44 patients who underwent LLTx according to the lung first principle, 35 patients were alive one year after transplantation. The one-year survival after combined LLTx with the lung first principle is 90,9%. The results of this meta-analysis are shown in figure 3A.
Two articles described patients who underwent combined LLTx where the liver was transplanted before the lung. [4,16] These results can also be found in table 1. In total there were 4 patients who had the liver transplanted before the lungs. We found no heterogeneity in year survival between the studies who transplanted the liver first, I2 = 0. All 4 patients who underwent LLTx with the liver first principle were alive one year after transplantation, the one-year survival is therefore 100%. The results of this meta-analysis are shown in figure 3B.
Five articles did not mention the used transplantation sequence

Figure 2 - A Forest plot of the meta-analysis comparing the proportion of one-year survival recorded in ten different articles. B Forest plot of the meta-analysis comparing the proportion of one-year survival recorded in seven different articles with a high quality score on the Rotterdam Medical Students Quality Scale.

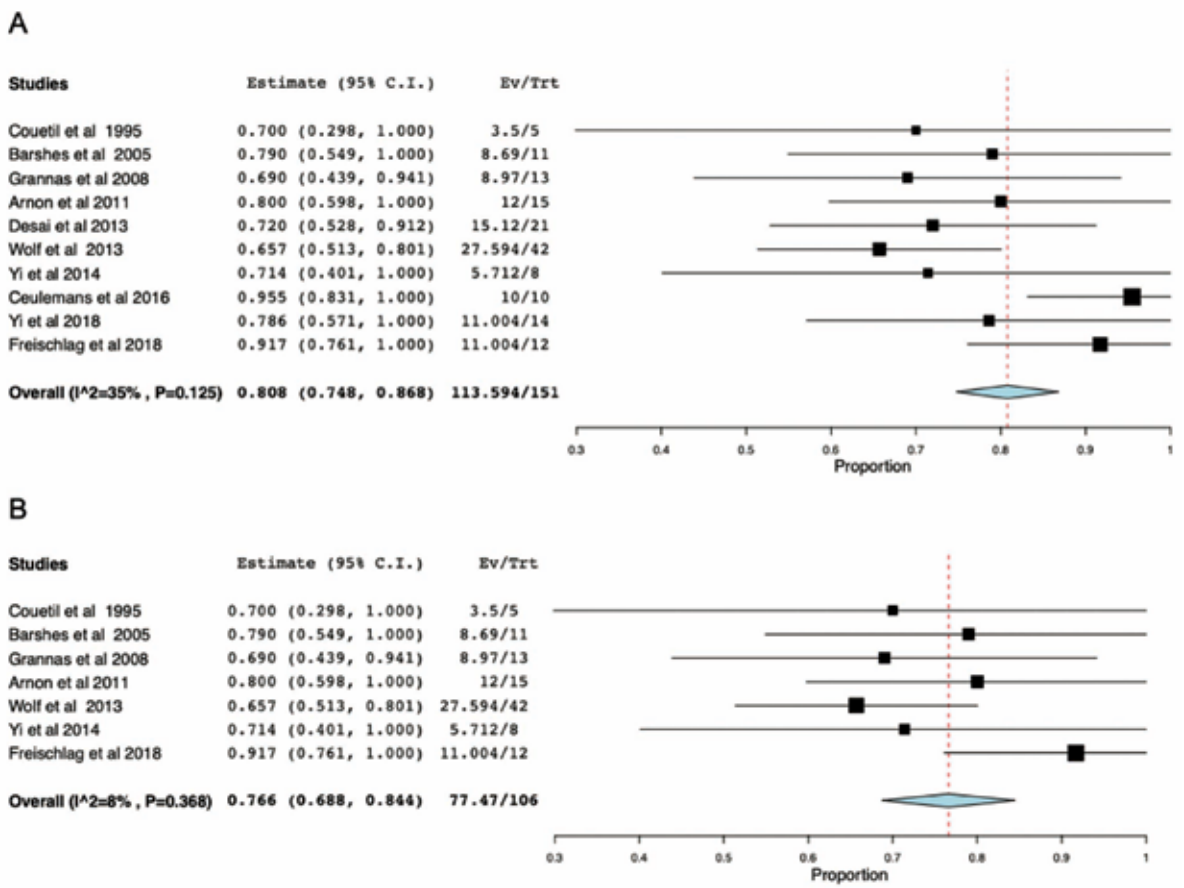


Figure 3 - A Forest plot of the meta-analysis comparing the proportion of one-year survival recorded in five different articles using an operation method of the lung first principle. B Forest plot of the meta-analysis comparing the proportion of one-year survival recorded in two different articles using an operation method of the liver first principle. C Forest plot of the meta-analysis comparing the proportion of one-year survival recorded in five different articles using an unclear operation method regarding the order of organ implantation

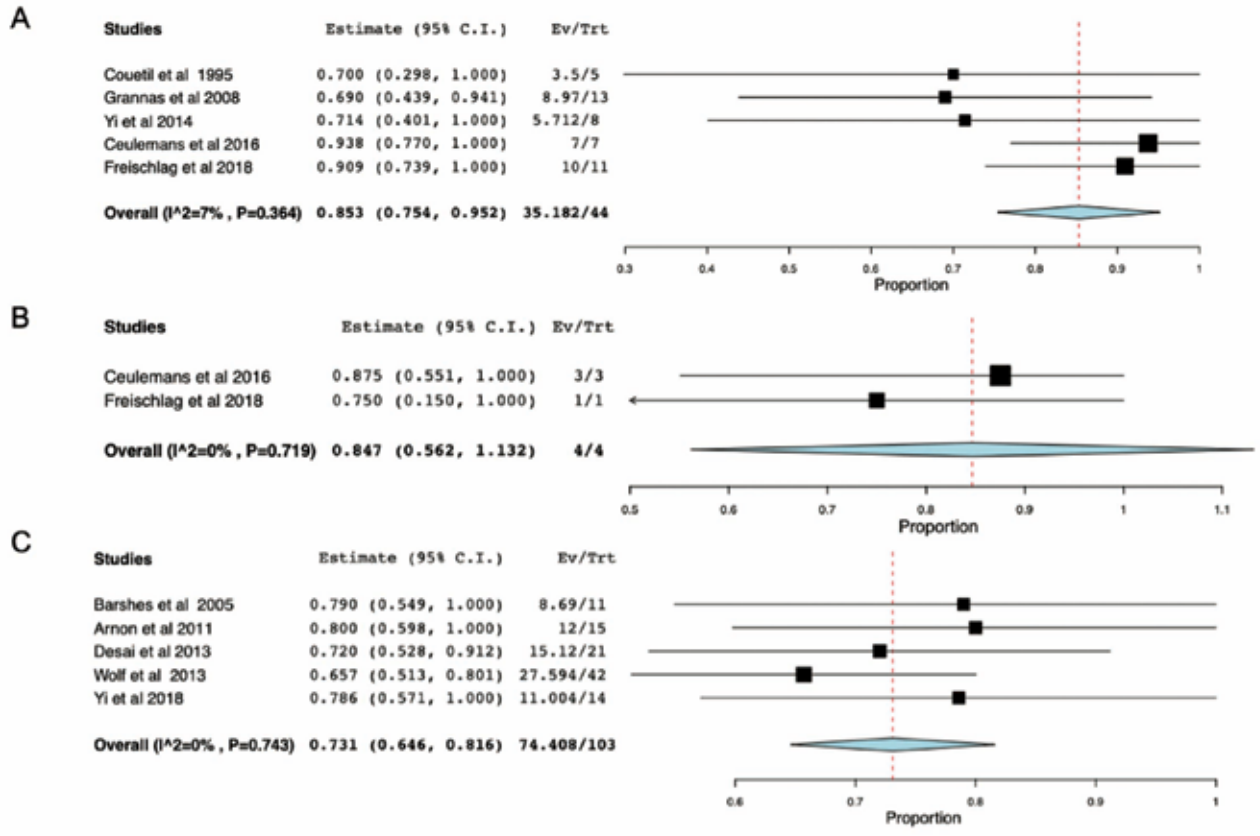
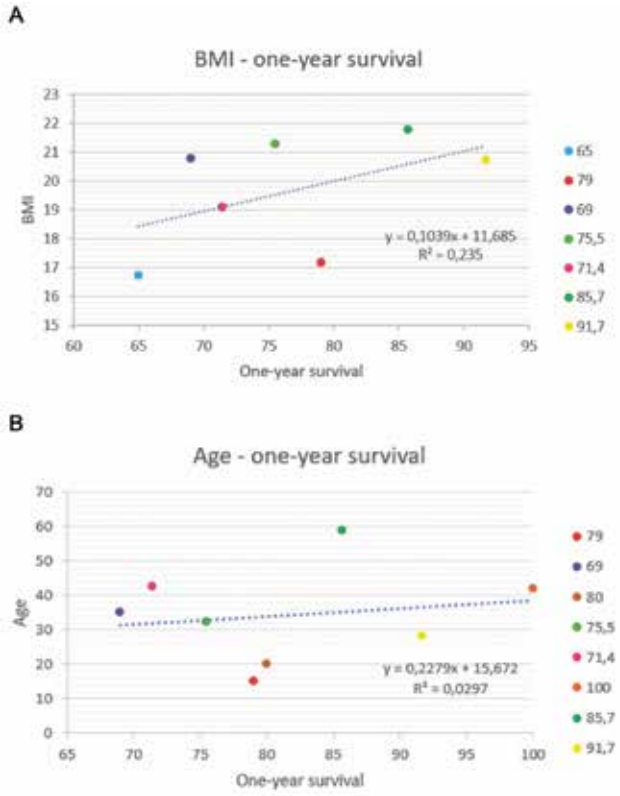


Figure 4 - A The correlation between transplant recipient BMI and one-year survival.
B The correlation between transplant recipient age and one-year survtival.



regarding which organ was first transplanted. [1,5,10,11,13] These results can also be found in table 1. We found no heterogeneity regarding the one-year survival between studies with unknown order of transplantation, I2 = 0. Of all the 103 patients whose operation method regarding the order of organ implantation were unclear, 74 patients are alive one year after transplantation. The one-year survival after an unclear sequence of operation is 72,2%. The results are shown in figure 3C.

Transplant recipient BMI

Seven articles mentioned transplant recipient BMI, see table 1. [1,5,12-16] Those seven articles are included in our secondarily analysis. Our results show that the highest mean transplant BMI found in our included studies is 21,8 and the lowest is 16,7. In figure 4A the correlation between the transplant recipient BMI and the one-year survival is shown. The correlation R2 is 0,235, which is not significant.

Transplant recipient age

Eight articles mentioned transplant recipient age, see table 1. [1,4,5,11,13-16] These eight articles were included in the secondarily analysis. The mean age of all eight included articles ranged from 15 to 58,8 years. In figure 4B the correlation between the recipient age and the one-year survival is shown. The correlation R2 is 0,0297, which is not significant.

Discussion/Conclusion

In our analysis, the one-year survival after combined LLTx is 75,2 %. With only those articles included with a high quality score, the one year survival after combined LLTx is 73,1%. The difference between our two analyses being 2,1%.

This is thus slightly lower than one-year survival after lung transplantation alone and liver transplantation alone, which are both 80,0%.[17,18] We can therefore conclude that the one-year survival after combined LLTx is lower.

The reason for this difference is unknown. One can speculate it might be because of patients who underwent combined LLTx were in worse condition before transplantation compared to patients who underwent lung- or liver transplantation alone. There could also be a difference in immunosuppressive medication and recovery time between the groups of combined and single transplantation. This should be further investigated.

Combined LLTx results in a 5% lower survival rate compared to lung or liver transplantation alone. This is only a slight difference. One can therefore conclude that combined LLTx is nearly as effective as single lung or liver transplantation. In addition, it seems the post-transplant complications are not significantly worse in combined LLTx compared to only a lung or liver transplant.

In this review the one-year survival was investigated because the individual articles used different endpoints, of which the one-year survival was most used.

One-year survival after combined LLTx with lung transplantation first is 90,9%. One-year survival after combined LLTx with liver transplantation first is 100%.

One-year survival after combined LLTx with unclear operation methods regarding the order of organ implantation is 72,2%.

In conclusion, there is 9,1% difference between the one-year survival of combined LLTx with the lung first principle, and combined LLTx with the liver first principle. However, there are only very limited data on the survival after the liver-first method (only 4 patients), so no definitive conclusions can be drawn.

Interestingly, the reported one-year survival in these studies is higher than the studies that do not mention the sequence (19-28% difference in one-year survival). The cause of this is unclear. Potentially, there is a bias towards newer studies reporting on the sequence, with potentially better survival. We can only speculate on this, and future research could further clarify this topic.

There is no clear correlation between the BMI and the one-year survival, because R2 = 0,235. This indicates that there is little relation between BMI and the one-year survival. The patient groups from Freischlag et al. [16] and Grannas et al. [14] have nearly the same mean recipient BMI's respectively 20,72 and 20,8 kg/m2, but very different one-year survival rates of respectively 91.7% and 69%. This also indicates that there is no clear statement that can be made regarding recipient BMI and one-year survival based on these data. The BMI range is from 16,7 to 21,8. The highest BMI is still a normal BMI, while in our population a lot of people are overweight and therefore have a BMI of 25 or higher. It struck us as odd that in our population the mean BMI was so low. For this reason, we suggest further studies to investigate the effect of BMI on the one-year survival

again, with a patient population that has more accurate BMI's compared to our western population.
The R2 = 0,0297 this shows that there is no clear correlation between the recipient age and the one-year survival either. The studies Yi et al. 2014 [15] and Ceulemans et al. [4] have nearly the same mean recipient age, respectively 42,5 and 42. However, the one-year survival rates are respectively 71,4% and 100%. These comparisons clearly show that transplant recipient survival is influenced by multiple factors.
Nevertheless, on both age and BMI only two articles were available, which created a limited patient population, therefore no conclusion can be drawn on the association between BMI and survival or age and survival. For this reason, further studies should investigate this topic.

Since we only included papers which were available at Erasmus MC, it could mean that we excluded articles with important data which may have influenced our results.
Additionally, because of the use of Major terms, it is possible that we missed articles that provided data on combined LLTx.
Furthermore, we excluded articles that had no clear mentioning of the one-year survival written in text. If the one-year survival was mentioned only in a figure, we excluded it to prevent inaccuracies. This could however have influenced our results.
We have also included 3 reviews, which do not contain original data. Due to limited time, we were not able to find other original studies used in these reviews. Since the data in reviews is not original, it could have interfered with the results. [5,11,13]
Lastly, over time patient survival has changed. This is caused by the better techniques of transplantation and experience. [19] We also included articles from more than 10 years ago, which could also have had an influence on our outcomes since we did not consider the different techniques used throughout the years.

We found that the Newcastle Ottawa Scale did not suit our sort of studies, even though some of them are cohort studies. This is because certain criteria from the scale were not applicable to our studies and the interventions that were conducted. We decided a follow up range of at least 5 years, because after 5 years 66-67% of the patients who received liver transplantations are alive. [18,20] For lung transplantation the 5-year survival is 54-66%. [17,21] This means that statistically, after 5 years, with at least 3 patients included in the studies, at least one of these patients would have died or would have underwent a different sort of adverse events. Therefore, there would be enough time and patients included in the studies to show a more long-term effect. [15,19]
Of all 151 combined LLTx patients, one patient received a combined heart-lung-liver transplant, Yi et al. [15] Another patient also received a islets of Langerhans transplantation, Yi et al. [14] We included these patients in our patient population, because there was no separation between the different transplantations in the articles. This could possibly have interfered with the results of our research. However, because these cases describe only two patients out of the 151 included in the studies, we can conclude that these patients did not have a significant impact on our results.

We were not able to investigate the cause of the difference between one-year survival in combined LLTx and single lung/liver transplantation. We recommend further studies to research the possible changes that could be made to limit the difference in survival. Furthermore, in this review, we only investigated the effect of transplant recipient BMI and age on the one-year survival, there are however multiple factors that could have an effect on the survival rates, which we did not investigate. For example, specific medication used after the transplantation and length of hospital stay. We suggest that further studies do investigate in these factors whether they affect the one-year survival after LLTx or not. Medication used and length of hospital stay where not distinctly mentioned in the articles of our research, so we were unable to investigate this. Lastly, we were not able to specify one-year survival per disease because the articles did not mention these numbers.

From our research we can conclude that the one-year survival after LLTx is 75,2%. Due to limited access to articles and information, we could not properly investigate whether liver or lung implantation first is better regarding the one-year survival. Furthermore, none of the investigated patient factors associated with one-year survival had a significant impact.

References

1. *Wolf JH, Sulewski ME, Cassuto JR, et al. Simultaneous thoracic and abdominal transplantation: can we justify two organs for one recipient? Am J Transplant 2013 July 01;13(7):1806-1816.*
2. *Health Resources and Services Administration, U S Department of Health & Human Services. Multiple Organ Transplants in the U.S. by Recipient ABO. 2019; Available at: https://optn.transplant.hrsa.gov/data/view-data-reports/national-data/#. Accessed 6/26, 2019.*
3. *United Network for Organ Sharing. Transplant trends. 2019; Available at: https://unos.org/data/transplant-trends/. Accessed 6/26, 2019.*
4. *Ceulemans LJ, Strypstein S, Neyrinck A, et al. Combined liver-thoracic transplantation: single-center experience with introduction of the 'Liver-first' principle. Transpl Int 2016 June 01;29(6):715-726.*
5. *Yi SG, Lunsford KE, Bruce C, et al. Conquering combined thoracic organ and liver transplantation: indications and outcomes for heart-liver and lung-liver transplantation. Curr Opin Organ Transplant 2018 April 01;23(2):180-186.*
6. *Salman J, Grannas G, Ius F, et al. The liver-first approach for combined lung and liver transplantation. Eur J Cardiothorac Surg 2018 December 01;54(6):1122-1127.*
7. *Nederlandse Transplantatiestichting. Hoe werkt orgaandonatie? Available at: https://www.transplantatiestichting.nl/medische-procedure/donatieprocedure/hoe-werkt-orgaandonatie. Accessed 6/26, 2019.*
8. *Wells, G A / Shea. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. 2018; Available at: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Accessed 12/17, 2018.*
9. *Newcombe RG. Interval estimation for the difference between independent proportions: comparison of eleven methods. Stat Med 1998 April 30;17(8):873-890.*
10. *Desai CS, Gruessner A, Habib S, et al. Survival of cystic fibrosis patients undergoing liver and liver-lung transplantations. Transplant Proc 2013 February 01;45(1):290-292.*
11. *Arnon R, Annunziato RA, Miloh T, et al. Liver and combined lung and liver transplantation for cystic fibrosis: analysis of the UNOS database. Pediatr Transplant 2011 May 01;15(3):254-264.*

Appendix

Appendix 1- Table 2

Table 2 - The Rotterdam Medical Students Quality Scale.

Terms of quality	Number of points
Studies have at least 3 patients who underwent combined lung-liver transplantation	1
Studies have mentioned inclusion and/or exclusion criteria	1
Studies have mentioned the characteristics of the patients included in the studies	1
Studies had a mean follow up of at least 5 years	1

Appendix 2 - Table 3

Table 3 - Overview of the quality score given to each study.

Article	Quality assessment	Explanation
Couetil et al 1995	3	1 point for correct number of patients included
		1 point for mentioning exclusion and/or inclusion criteria
		1 point for mentioning patient characteristics
Barshes et al 2005	3	1 point for correct number of patients included
		1 point for mentioning exclusion and/or inclusion criteria
		1 point for mentioning patient characteristics
Grannas et al 2008	3	1 point for correct number of patients included
		1 point for mentioning patient characteristics
		1 point for a mean follow up of at least 5 years
Arnon et al 2011	3	1 point for correct number of patients included
		1 point for mentioning exclusion and/or inclusion criteria
		1 point for mentioning patient characteristics
Desai et al 2013	2	1 point for correct number of patients included
		1 point for mentioning exclusion and/or inclusion criteria
Wolf et al 2013	3	1 point for correct number of patients included
		1 point for mentioning exclusion and/or inclusion criteria
		1 point for patient characteristics
Yi et al 2014	3	1 point for correct number of patients included
		1 point for mentioning exclusion and/or inclusion criteria
		1 point for mentioning patient characteristics
Ceulemans et al 2016	2	1 point for correct number of patients included
		1 point for mentioning patient characteristics
Yi et al 2018	1	1 point for correct number of patients included
Freischlag et al 2018	3	1 point for correct number of patients included
		1 point for mentioning exclusion and/or inclusion criteria
		1 point for mentioning patient characteristics

12. *Couetil JP, Houssin DP, Soubrane O, et al. Combined lung and liver transplantation in patients with cystic fibrosis. A 4 1/2-year experience. J Thorac Cardiovasc Surg 1995 November 01;110(5):1415-22; discussion 1422.*
13. *Barshes NR, DiBardino DJ, McKenzie ED, et al. Combined lung and liver transplantation: the United States experience. Transplantation 2005 November 15;80(9):1161-1167.*
14. *Grannas G, Neipp M, Hoeper MM, et al. Indications for and outcomes after combined lung and liver transplantation: a single-center experience on 13 consecutive cases. Transplantation 2008 February 27;85(4):524-531.*
15. *Yi SG, Burroughs SG, Loebe M, et al. Combined lung and liver transplantation: analysis of a single-center experience. Liver Transpl 2014 January 01;20(1):46-53.*
16. *Freischlag KW, Messina J, Ezekian B, et al. Single-Center Long-Term Analysis of Combined Liver-Lung Transplant Outcomes. Transplant Direct 2018 April 26;4(5):e349.*
17. *Thabut G, Mal H. Outcomes after lung transplantation. J Thorac Dis 2017 August 01;9(8):2684-2691.*
18. *Jain A, Reyes J, Kashyap R, et al. Long-term survival after liver transplantation in 4,000 consecutive patients at a single center. Ann Surg 2000 October 01;232(4):490-500.*
19. *Polak WG, Peeters PM, Slooff MJ. The evolution of surgical techniques in clinical liver transplantation. A review. Clin Transplant 2009 September 01;23(4):546-564.*
20. *Nederlandse Transplantatiestichting. Hoe lang gaat een donorlever mee? Available at: https://www.transplantatiestichting.nl/cijfers/hoe-lang-gaat-een-donororgaan-mee/hoe-lang-gaat-een-donorlever-mee. Accessed 12/17, 2018.*
21. *Nederlandse Transplantatiestichting. Hoe lang gaan donorlongen mee? Available at: https://www.transplantatiestichting.nl/cijfers/hoe-lang-gaat-een-donororgaan-mee/hoe-lang-gaan-donorlongen-mee. Accessed 12/17, 2018.*

The prognostic value of Next-Generation Sequencing for the detection of molecular Minimal Residual Disease in Acute Myeloid Leukemia

a systematic review and meta-analysis

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Abstract

Objective: **The aim of this article is to evaluate the prognostic value of molecular minimal residual disease (MRD) detection by next-generation sequencing (NGS) to predict relapse in acute myeloid leukemia (AML).**

Methods: **We conducted a systematic review using the PubMed database and performed a meta-analysis. Outcome measures were cumulative incidence of relapse (CIR), relapse free survival (RFS) and overall survival (OS).**

Results: **After screening, we included 6 articles that met our inclusion criteria. The inclusion criteria were as followed: patients with (primary) AML, >18 years, reached complete remission (CR) after consolidation and MRD-detection after CR .The search terms that were used were AML, MRD and NGS. The conducted meta-analysis and the articles included in the systematic review show that NGS-based positive MRD detection is related to an increased CIR (HR 3.38, 95% CI [1.71, 6.65], P=0.0004), reduced RFS (HR 2.54, 95% CI [1.65, 3.93], P<0.0001) and reduced OS (HR 2.25, 95% CI [1.76, 2.88], P<0.00001) in patients with AML. In 90% of the AML patients in complete remission at least one mutation is detected, which can serve as a marker for residual disease.**

Conclusion: **Next-generation sequencing is of prognostic value to detect minimal residual disease in AML patients to predict the risk of relapse. Detection of MRD might be used to adjust post-remission therapy for the purpose of lowering the relapse rate after having received complete remission with intensive induction chemotherapy.**

Keywords: **minimal residual disease, next-generation sequencing, acute myeloid leukemia, relapse, prognosis.**

Introduction

Acute myeloid leukemia (AML) is characterized by an increase in the number of myeloid cells (blasts) in the bone marrow and an arrest in maturation of the hematopoietic cells.[1] AML is a genetically heterogeneous disease composed of multiple clones that are characterized by combinations of somatic molecular mutations.[2] Over the past decade, refinements in the diagnosis of subtypes of AML and advances in therapeutic approaches have improved the prospects of patients with AML.[1] More than 70% of the patients with AML attain morphologic complete remission (CR) with standard induction chemotherapy.[3] Although the outcome of patients with AML has improved, relapse continues to represent the leading cause of death in the majority (>50%) of patients in complete remission.[4,5] Therefore, molecular markers to predict relapse are urgently needed to tailor therapy.

Minimal residual disease can be used to determine the risk of relapse.[2,6-10] Minimal residual disease (MRD) is the number of aberrant cells left after therapy when a patient is in complete remission (CR, <5% leukemic cells morphologically visible in bone marrow or in peripheral blood).[11] Therapy adjustments after MRD detection can be made to prevent relapse in AML patients. Classical MRD techniques are flow cytometry and RQ-PCR of fusion transcript [12], these techniques are currently being used in clinical practice.[13] The use of next-generation sequencing for the detection of MRD is upcoming. Next-generation sequencing (NGS) is a fast technique to sequence many molecular variants.[14] Advantages of NGS are standardization of data formats and the possibility to sequence multiple genes at the same time, contrary to flow cytometry and RQ-PCR.[15] The aim of this article is to evaluate whether NGS is a suitable technique to predict relapse in AML patients by measuring MRD.

Methods

Search strategy

On December 18th, 2018, we conducted a search in the PubMed electronic database for articles using Medical Subject Headings terms (MeSH) and title/abstract terms (tiab) for acute myeloid

leukemia (AML), minimal residual disease (MRD) and next-generation sequencing (NGS). We also used synonyms for these terms to prevent exclusion of relevant studies (see Appendix for the whole search query). We included articles for screening if they cite the previous mentioned terms in the title and/or abstract.

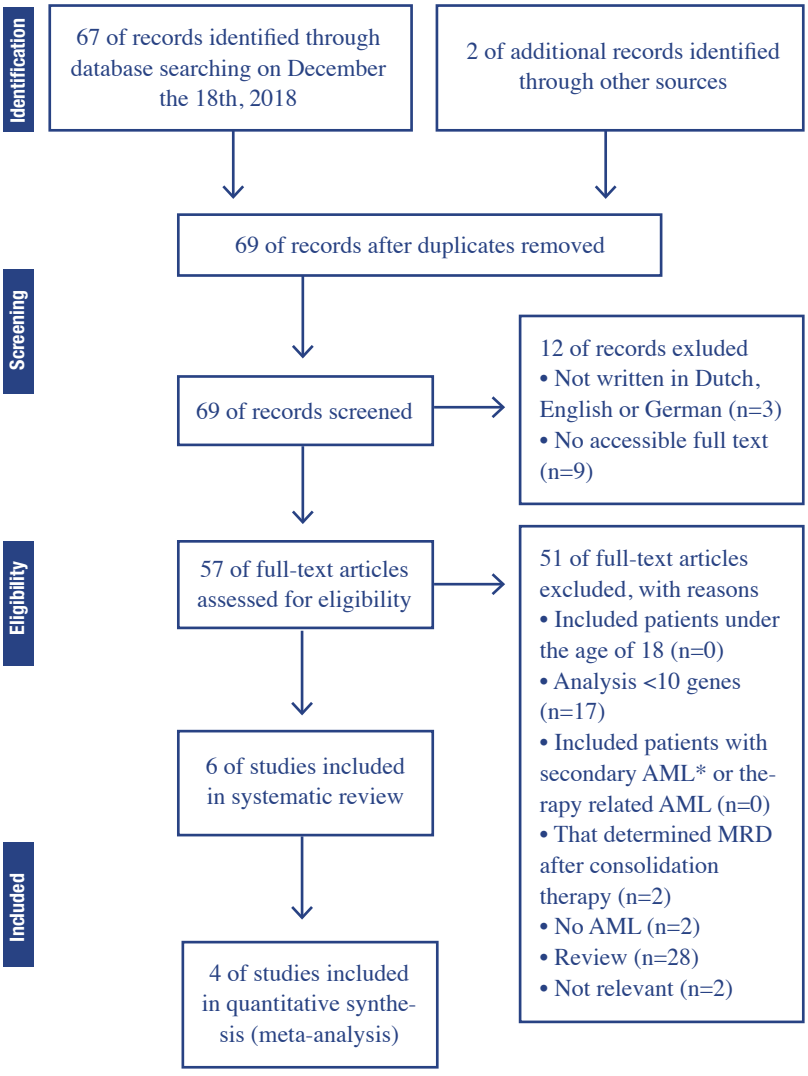
Selection criteria

At first, we excluded articles written in other languages than Dutch, English and German from any further screening. We also excluded articles that were not available as full text (Free and Erasmus MC). Three authors screened all remaining titles and abstract independently for eligibility based on the following exclusion criteria: articles that (1) included patients under the age of 18; (2) included patients that were not in complete remission; (3) conducted analysis of less than 10 (mutated) genes*; (4) determined MRD after consolidation therapy; (5) included patients with secondary AML** or (chemo)therapy related AML; (6) included others forms of acute leukemia (such as acute lymphoblastic leukemia (ALL) and acute promyelocytic leukemia (APL); (7) are a review and (8) were not relevant to obtain our objective. Secondly, we did a full-text analysis of the remaining and potentially relevant articles. After this phase, discrepancies between the authors were discussed to achieve consensus considering the inclusion of the articles.

*AML is a heterogeneous disease and therefore a large panel is needed to determine the risk of relapse.

**Secondary AML: a form of AML arising from a previous clonal disorder (such as myelodysplastic syndrome).[16]

Figure 1- Flowchart of search strategy and exclusion criteria.



Outcome measures

The outcomes of this systematic review and meta-analysis are cumulative incidence of relapse (CIR), the relapse free survival (RFS) and the overall survival (OS). Relapse is defined as presence of >5% myeloid blasts on a bone marrow aspirate or in peripheral blood.[2] CIR is defined from the date of CR to the date of relapse, adjusted for death as a competing event. RFS is defined from the date of CR to the date of relapse or death, whichever comes first. OS is defined as the date of CR to the date of death from any cause.[8]

Meta-analysis

We performed a statistical analysis on the effect of MRD detection with NGS on CIR, RFS and OS. We used Review manager 5.3 (Cochrane Library) to conduct the statistical analysis. A random effects model was used to construct forest plots with a 95% confidence interval. Beforehand we calculated standard errors (SE) using the following formula adapted from the handbook of Cochrane [28]:

((Ln(upper limit CI)- Ln(lower limit CI)))/3,92=SE

Statistical heterogeneity was assessed using I-square test for heterogeneity and was considered significant if I2 >30%).[17]

Results

Study selection

With the search strategy described before, we identified a total of 67 articles. Of these 67 articles, 9 were excluded due to unavailability of full-texts and 3 articles were excluded since they were not written in Dutch, English or German. After screening title and abstract of the remaining 55 articles, 51 articles were further excluded based on the exclusion criteria (see Figure 1). We also included two articles found through another source, since both articles were not appropriately indexed in PubMed. The first article [9] was found through the references of an editorial. [18] The second article [8] was found through the option 'similar articles' in Pubmed. This resulted in a total of 6 articles, which met the eligibility criteria.

Study characteristics

All included studies were retrospective cohort studies. The sample size varied from 50 to 482 patients. All patients had Acute Myeloid Leukemia, with different types of mutations in the bone marrow. These different types of mutations lead to a different outcome and prognosis[29]. The age of patients differed from 18 [6,7,10] to 84 years[6] and the median age differed from

Table 1 - Study characteristics of the included studies

Author	Study design	Location	Sample size	Age	Next Generation Sequencing (NGS)	Gene panels	Taking age related clonal hematopoiesis into account	Follow-up	Consolidation therapy	Outcome
Jongen-Lavrencic et al. (2018)	Cohort study	Netherlands	430	51 (18-65) (median)	Targeted NGS	54 genes	Yes	4 years (median)	According to protocol*	CIR, RFS and OS
Klco et al. (2015)	Cohort study	USA	50	50,8 (mean)	Whole-genome and targeted NGS	50 genes with targeted sequencing	No	Minimum of 1 year	According to protocol*	RFS and OS
Getta et al. (2017)	Cohort study	USA	83	58 (21-78) (median)	Targeted NGS	28 genes	No	13 months (median)	According to protocol*	CIR and OS
Hirsch et al. (2017)	Cohort study	France	69	58 (18-84) (median)	Targeted NGS	122 genes	Yes	24 months (median)		RFS and OS
Thol et al. (2018)	Cohort study	Germany	116	51 (median) 51.0	Error-corrected NGS	24 genes	Yes	6 years (median)	According to protocol* only alloSCT	CIR, RFS and OS
Morita et al. (2018)	Cohort study	USA	131	(39.0-55.0) (median)	Targeted NGS	35 genes	Yes	35 months (median)	According to protocol*	CIR, RFS and OS

Abbreviations: VAF, Variant Allel Frequency; AutoSCT, Autologous stem cell transplantations; AlloSCT, Allogeneic stem cell transplantations; CIR, Cumulative incidence of relapse; RFS, Relapse free survival; OS, Overall Survival
*According to protocol includes autoSCT/chemotherapy or alloSCT only

50,8 [10] to 58.[6] The consolidation therapies consisted of hematopoietic stem cell transplantation (HSCT), chemotherapy or no consolidation therapy at all and differed per study., Different gene panels were used in each study for targeted sequencing. Table 1 shows the characteristics of all included studies. Furthermore, 4 studies measured MRD after induction therapy [7-10], whereas 2 studies measured MRD after induction and consolidation therapy.[2,6]

All mutations

In most studies in ≥90% of the AML patients in complete remission at least one mutation can be detected with NGS.[6,7,9] In order to use NGS as a reliable method to measure minimal residual disease, some studies made a distinction between somatic mutations and age-related mutations representing respectively residual leukemia (non-DTA mutations) and DTA mutations (DNMT3A, TET2 and ASXL1). Some studies did not make the previously mentioned distinction.[2,9] Yet all studies have shown significant differences in the outcome measures CIR, RFS and OS (see Meta-analysis). There hasn't been made a clear distinction yet between age-related mutations and pre-leukemic mutations.

Age-related clonal hematopoiesis

Mutations accumulate in clonally derived hematopoietic cells in individuals with no apparent hematological disease as a result of aging.[20] The persistence of mutations that are most commonly associated with age-related clonal hematopoiesis (i.e. DTA mutations) during complete remission did not contribute to a measurable risk of relapse.[7] Eventually, the risk to develop hematological malignancies is 0,5% per year.[21] Nevertheless, according to a study conducted by Jaiswal et al [21] age-related mutations at diagnosis are very exceptional in patients younger than 40 years. Between 70-79 years the frequency of age-related mutations is 11,7% (8.6-15.7%).[21]

Non-DTA mutations

Mutations other than the DTA mutations that occasionally persisted during morphologic complete remission (CR) are associated with an increased CIR, reduced RFS and reduced OS.[7] Patients with two or more mutations during CR after treatment, had a reduced RFS (HR 0,109, p=0,0006) and reduced OS (HR 0,071, p=0,006) in comparison with patients with zero or one mutations.[6]

Meta-analysis

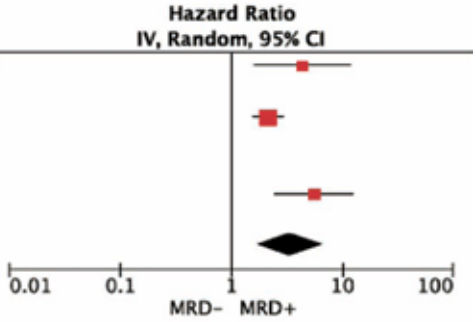
Statistical analysis of CIR (n=519), RFS (n=486) and OS (n=569) showed significant hazard ratios for each outcome (CIR: HR 3.38, 95% CI [1.71, 6.65], P=0.0004, I2=66%, (See Figure 2, appendix); RFS: HR 2.54, 95% CI [1.65, 3.93], P<0.0001 I2=52% (See Figure 3, appendix); OS: HR 2.25, 95% CI [1.76, 2.88], P<0.00001, I2=0% (See Figure 4, appendix)). These results demonstrate an association between MRD-detection with NGS and an increased CIR, reduced RFS and reduced OS. The articles that were not included in the meta-analysis had no sufficient data to calculate the hazard ratios.

Heterogeneity for the CIR and RFS is >30% and thus present. For this reason, it was appropriate to conduct a random effects model. Heterogeneity for the OS is <30% and thus absent. Therefore, a fixed effects model could be used to conduct the analysis. However, there was no change in using the fixed effects model in comparison with the random effects model, and hence we used the random effects to conduct the final analysis.

Figure 2: Effect of MRD positivity detected with NGS on Cumulative Incidence of Relapse (CIR)

Study or Subgroup	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Random, 95% CI
Getta et al. (2017)	1.47017584	0.50061864	24.6%	4.35 [1.63, 11.60]
Hirsch et al. (2017) (1)	0	0		Not estimable
Jongen-Lavrencic et al. (2018)	0.76080583	0.15741772	46.0%	2.14 [1.57, 2.91]
Klco et al. (2015) (1)	0	0		Not estimable
Morita et al. (2018) (1)	0	0		Not estimable
Thol et al. (2018)	1.71918878	0.41568333	29.3%	5.58 [2.47, 12.60]
Total (95% CI)			**100.0%**	**3.38 [1.71, 6.65]**

Heterogeneity: $Tau^2 = 0.24$; $Chi^2 = 5.92$, df = 2 (P = 0.05); $I^2 = 66\%$
Test for overall effect: Z = 3.52 (P = 0.0004)

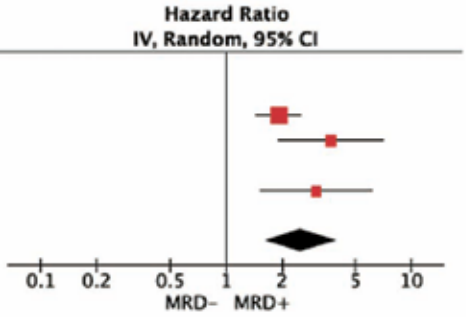


Footnotes
(1) Not included in meta-analysis

Figure 3: Effect of MRD positivity detected with NGS on Relapse Free Survival (RFS)

Study or Subgroup	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Random, 95% CI
Getta et al. (2017) (1)	0	0		Not estimable
Hirsch et al. (2017)(1)	0	0		Not estimable
Jongen-Lavrencic et al. (2018)	0.65232519	0.14054001	49.7%	1.92 [1.46, 2.53]
Klco et al. (2015)	1.30019166	0.33095996	26.1%	3.67 [1.92, 7.02]
Morita et al. (2018) (1)	0	0		Not estimable
Thol et al. (2018)	1.11841492	0.351984	24.2%	3.06 [1.54, 6.10]
Total (95% CI)			**100.0%**	**2.54 [1.65, 3.93]**

Heterogeneity: $Tau^2 = 0.08$; $Chi^2 = 4.20$, df = 2 (P = 0.12); $I^2 = 52\%$
Test for overall effect: Z = 4.21 (P < 0.0001)

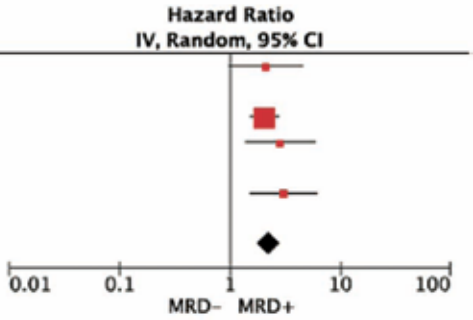


Footnotes
(1) Not included in meta-analysis

Figure 4: Effect of MRD positivity detected with NGS on Overall Survival (OS)

Study or Subgroup	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Random, 95% CI
Getta et al. (2017)	0.74193734	0.39428225	10.1%	2.10 [0.97, 4.55]
Hirsch et al. (2017) (1)	0	0		Not estimable
Jongen-Lavrencic et al. (2018)	0.72270598	0.15493144	65.6%	2.06 [1.52, 2.79]
Klco et al. (2015)	1.05082162	0.36792169	11.6%	2.86 [1.39, 5.88]
Morita et al. (2018) (1)	0	0		Not estimable
Thol et al. (2018)	1.11841492	0.35364652	12.6%	3.06 [1.53, 6.12]
Total (95% CI)			**100.0%**	**2.25 [1.76, 2.88]**

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 1.54$, df = 3 (P = 0.67); $I^2 = 0\%$
Test for overall effect: Z = 6.47 (P < 0.00001)



Footnotes
(1) Not included in meta-analysis

Appendix

("Neoplasm, Residual"[Mesh] OR "minimal residual disease"[tiab] OR "measurable residual disease" [tiab] OR "MRD"[tiab]) AND ("Leukemia, Myeloid, Acute"[Mesh] OR "acute myeloid leukemia"[tiab] OR "AML"[tiab]) AND ("High-Throughput Nucleotide Sequencing"[Mesh] OR "Next generation sequencing"[tiab] OR "whole exome sequencing" [tiab] OR "whole exome sequencing" [Mesh] OR "whole genome sequencing" [tiab] OR "exome sequencing" [tiab] OR "massively parallel sequencing" [tiab] OR "NGS"[tiab]).

Discussion/conclusion

In this study we investigated the prognostic value of MRD in AML measured by NGS. After searching
through the PubMed library and other sources, we included 6 articles that were relevant for our research question. With these three outcomes we performed a meta-analysis on the effect of NGS-detected MRD on relapse.

In order to assess the prognostic value of NGS to detect MRD in AML patients three outcomes were used: cumulative incidence of relapse, relapse free survival and overall survival. The studies assessing CIR showed a significant increased CIR if MRD was detected with NGS. The studies assessing RFS and OS showed a

significant reduction in RFS and OS if MRD was detected with NGS. Thus, NGS is of prognostic value to detect the MRD status of AML patients to predict relapse.

Clinical relevance

In 90% of the AML patients in complete remission at least one mutation is detected, which can serve as a residual disease marker.[8] The detection of MRD in AML is of clinical relevance, since MRD positivity or negativity predicts relapse and therefore might be used to adjust therapy. Flow cytometry is currently standard for the detection of MRD.[13] A recent study suggests that the analysis of information acquired through flow cytometry to determine MRD is a difficult task that requires precise experience and is therefore difficult to standardize.[22] In contrast to this, NGS can be standardized. A disadvantage of NGS is the limited standardization.[19]

Limitations of NGS in clinical practice

We established three matters that still need to be improved before NGS can be implemented for clinical use. These three matters are the gene panels, the depth of sequencing and clonal hematopoiesis.

AML is a heterogeneous disease and because of this it is important to use a gene panel with mutations that are relevant to the pathogenesis of AML. All of the included studies composed a gene panel by identifying different genes. This could cause discordant results. However, all studies showed significant outcomes. This probably means that all studies used the most important and most frequently mutated genes in AML.

Secondly the depth of sequencing differs between the studies. Sequencing depth is related to the form of NGS. Used forms of NGS in the studies were targeted NGS and whole genome sequencing. Whole genome sequencing can be used to identify mutations anywhere in the genome (coding and non-coding regions), whereas targeted NGS can be used to identify predetermined mutations.[23,24] Targeted sequencing has a greater depth than whole genome sequencing, resulting in a higher sensitivity.[24]

Furthermore, when large numbers have to be sequenced with NGS, errors often occur in the reads.[25] To prevent these errors, Thol et al [10] used a form of error-corrected NGS, leading to fewer errors in the reads. Schmitt et al [26] has demonstrated that ~ 99% of sequencing errors are corrected with error-corrected NGS.[26] Using error-corrected NGS can improve the sequencing depth, also leading to a higher sensitivity. However, we found that there was no significant difference between error-corrected NGS and other forms of NGS without error-correction in terms of CIR, RFS and OS. There is no consensus yet about whether error-corrected NGS should be implemented.

Lastly, some of the studies [6-8,10] left the mutations related to clonal hematopoiesis (DTA mutations) out of their analysis and some [2,9] did not. DTA mutations can appear in healthy individuals, without any sign of hematological malignancies [27]. These age-related mutations are probably related to the early development of hematologic cancers.[21] Nonetheless, the risk of developing a hematologic malignancy is 0,5% per year.[21]

Including DTA mutations in the NGS based MRD-analysis can lead to distorted outcomes. However, the results are probably an underestimation of the actual outcome, since including DTA mutations already lead to significant outcomes. Studies [7] show that clonal hematopoiesis is not related to the risk of relapse in a follow-up time of 4 years.

Limitations of this systematic review

We have the awareness that our study could be biased because of the limited publications we were able to find. The included studies in this article proved that NGS for detecting MRD in AML patients is prognostic in comparison to MRD negative patients. There is a possibility that unpublished studies have found no prognostic value with NGS for detecting MRD in AML patients. So, there could be publication bias. Nevertheless, NGS is a recently introduced technique to detect MRD in AML and therefore more studies will follow in the near future.

Next-generation sequencing is of prognostic value to detect minimal residual disease in acute myeloid leukemia. MRD detection by NGS improves the prediction of the risk of relapse and could therefore be used to adjust consolidation therapy in patients with AML. For further research, a consensus for the use of MRD-specific gene panel has to be reached. Furthermore, prospective studies with bigger cohorts need to be performed to determine whether therapy adjustment after MRD determination with NGS will actually lead to lower relapse rates. Finally, mutations associated to the age-related clonal hematopoiesis need to be further determined.

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References

1. *Lowenberg B. Acute Myeloid Leukemia. 1999;341:1051-1062.*
2. *Getta, Bartlomiej M. Devlin, Sean M. Levine, et al. Multicolor Flow Cytometry and Multigene Next-Generation Sequencing Are Complementary and Highly Predictive for Relapse in Acute Myeloid Leukemia after Allogeneic Transplantation. Biology of Blood and Marrow Transplantation 2017;23:1064-1071.*
3. *Franco Mandelli, Marco Vignetti, Stefan Suciu, et al. Daunorubicin Versus Mitoxantrone Versus Idarubicin As Induction and Consolidation Chemotherapy for Adults With Acute Myeloid Leukemia: The EORTC and GIMEMA Groups Study AML-10. Journal of Clinical Oncology 2009 Nov 10;27:5397-5403.*
4. *Burnett AK. Acute myeloid leukemia: treatment of adults under 60 years. Reviews in clinical and experimental hematology 2002 Mar;6:26.*
5. *Walter RB, Othus M, Burnett A, Löwenberg B, et al. Resistance prediction in AML: analysis of 4601 patients from MRC/NCRI, HOVON/SAKK, SWOG and MD Anderson Cancer Center. Leukemia 2015 Jan 1,;29:312-320.*
6. *Hirsch P, Tang R, Abermil N, et al. Precision and prognostic value of clone-specific minimal residual disease in acute myeloid leukemia. Haematologica 2017 Jul;102:1227-1237.*
7. *Jongen-Lavrencic M, Grob T, Hanekamp D, et al. Molecular Minimal Residual Disease in Acute Myeloid Leukemia. The New England journal of medicine 2018 Mar 29,;378:1189-1199.*
8. *Morita K, Kantarjian HM, Wang F, et al. Clearance of somatic mutations at remission and the risk of relapse in acute myeloid leukemia. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2018 Jun 20,;36:1788-1797.*

9. *Klco JM, Miller CA, Griffith M, et al. Association Between Mutation Clearance After Induction Therapy and Outcomes in Acute Myeloid Leukemia. JAMA 2015 Aug 25,;314:811-822.*
10. *Thol F, Gabdoulline R, Liebich A, et al. Measurable residual disease (MRD) monitoring by NGS before allogeneic hematopoietic cell transplantation in AML. Blood 2018 Sep 6,;132:1703-1713.*
11. *Roland B. Walter, Hagop M. Kantarjian, et al. Effect of Complete Remission and Responses Less Than Complete Remission on Survival in Acute Myeloid Leukemia: A Combined Eastern Cooperative Oncology Group, Southwest Oncology Group, and M. D. Anderson Cancer Center Study. Journal of Clinical Oncology 2010 Apr 1,;28:1766-1771.*
12. *Dongen J, Velden V, Brüggemann M, et al. Minimal residual disease diagnostics in acute lymphoblastic leukemia: Need for sensitive, fast, and standardized technologies. Blood 2015 Jun 25,;125:3996-4009.*
13. *HOVON 135 AML / SAKK.*
14. *Behjati S, Tarpey PS. What is next generation sequencing? Archives of disease in childhood. Education and practice edition 2013 Dec;98:236-238.*
15. *Endrullat C, Glökler J, Franke P, et al. Standardization and quality management in next-generation sequencing. Applied & Translational Genomics 2016 Sep;10:2-9.*
16. *Szotkowski T, Rohon P, Zapletalova L, et al Secondary acute myeloid leukemia a single center experience. Neoplasma 2010;57:170-178.*
17. *9.5.2 Identifying and measuring heterogeneity. Available at: https://handbook-5-1.cochrane.org/chapter_9/9_5_2_identifying_and_measuring_heterogeneity.htm.*
18. *Pastore F, Levine RL. Next-Generation Sequencing and Detection of Minimal Residual Disease in Acute Myeloid Leukemia: Ready for Clinical Practice? JAMA 2015 Aug 25,;314:778-780.*
19. *Ravandi F, Walter RB, Freeman SD. Evaluating measurable residual disease in acute myeloid leukemia. Blood advances 2018 Jun 12,;2:1356.*
20. *Welch J, Ley T, Link D, et al. The Origin and Evolution of Mutations in Acute Myeloid Leukemia. Cell 2012 Jul 20,;150:264-278.*
21. *Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. The New England journal of medicine 2014 Dec 25,;371:2488-2498.*
22. *Brooimans RA, van der Velden, Vincent H.J, Boeckx N, et al. Immunophenotypic measurable residual disease (MRD) in acute myeloid leukemia: Is multicentric MRD assessment feasible? Leukemia Research 2019 Jan;76:39-47.*
23. *Whole-Genome Sequencing. Available at: https://www.illumina.com/techniques/sequencing/dna-sequencing/whole-genome-sequencing.html.*
24. *Targeted Gene Sequencing Panels. Available at: https://www.illumina.com/techniques/sequencing/dna-sequencing/targeted-resequencing/targeted-panels.html.*
25. *Pfeiffer F, Gröber C, Blank M, et al. Systematic evaluation of error rates and causes in short samples in next-generation sequencing. Scientific reports 2018 Jul 19,;8:10950-14.*
26. *Michael W. Schmitt, Scott R. et al. Detection of ultra-rare mutations by next-generation sequencing. Proceedings of the National Academy of Sciences of the United States of America 2012 Sep 4,;109:14508-14513.*
27. *Genovese G, Kähler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. The New England journal of medicine 2014;371:2477.*
28. *7.7.7.2 Obtaining standard errors from confidence intervals and P values: absolute (difference) measures Available at: https://handbook-5-1.cochrane.org/chapter_7/7_7_7_2_obtaining_standard_errors_from_confidence_intervals_and.htm*
29. *[1] DiNardo CD, Cortes JE. Mutations in AML: prognostic and therapeutic implications. Hematology. American Society of Hematology. Education Program 2016 Dec 2,;2016:348-355.*

The clinical effect of Impella on Right Ventricular Failure

a systematic review

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Abstract

Introduction: **Right ventricular failure (RVF) is an independent risk factor for overall mortality. Therefore, proper treatment to support the right ventricle is of major importance, a temporary supportive device for the right ventricle (Impella RP or RD) may be an option.**
Objective: **The aim of this article is to review the physiological effects and outcome of the Impella RP or RD in patients with RVF.**
Methods: **We conducted a literature search on PubMed, until December 23th, about the effect of Impella RD or RP on right ventricular failure. Inclusion was based on English articles with more than 5 patients describing the effect of a right sided Impella when right heart dysfunction was present. We analysed the 30-days survival, Cardiac Index (CI) and Central Venous Pressure (CVP) among other things.**
Results: **Three studies showed an increase in Cardiac Index. Three studies showed a decrease in Central Venous Pressure. Death was a major complication of Impella RP and RD, which differed between 0,0% and 87,5%.**
Discussion and conclusions: **Our study outcome suggests that Impella RP and RD are adequate supportive devices in right ventricular failure. However our results show that the Impella should not be used clinically until its mechanical reliability is improved.**

Introduction

Right Ventricular Failure (RVF) is a very deadly disease with a mortality up to 75%[1,2]. RVF has many origins. The primary causes of RVF are Acute Myocardial Infarction (AMI), Pulmonary Embolism (PE) and valvular dysfunction. Secondary, RVF can be caused postprocedural, such as post-cardiotomy, post-transplant and post LVAD.[3-5] It is shown that RVF is an independent risk factor for overall mortality and cardiovascular mortality[6].

The treatment of RVF mainly consists of optimizing the RV preload, reducing the RV afterload and improving the myocardial pump function[7-9]. At first, the RV preload should be optimized by managing the fluid status[9]. Furthermore, the right ventricular contractility can be improved by inotropes, such as dobutamine[7,10]. At last, several strategies exist to reduce the afterload such as nitrodilators and hydralazine[8]. The surgical procedures used to treat RVF are mainly based on treating the cause of RVF, such as valvular replacement[5,11].

In addition, new possibilities emerge, of which mechanical temporary support is one. Impella RD (Abiomed Inc, Danvers, MA) is a microaxial flowpump which is designed for short term use, with a maximum of ten days. The device consists of three parts: a pump, a mobile consile which is connected to the pump with the driveline of the catheter and a purger, which is the catheter with a driveline and a purgeline. The Impella RD is implanted through a sternotomy.[3,12]

Impella RP (Abiomed Inc, Danvers, MA) has the advantage that it can be placed percutaneously[3].

Acute RV dysfunction can induce LV dysfunction[13]. There are several mechanism why RV failure can lead to LV failure. First, the impaired RV contraction leads to a reduced transpulmonary blood flow and a reduced filling of the left atrium. Second, the RV dilation can lead to a deviation of the interventricular septum to the left, which causes a decrease in the left ventricular compliance. If these effects occur collaboratively, the LV diastolic filling drops and that will lead to reduced stroke volume and cardiac output.[14] It is implied that the RV has a greater capability for rapid recovery than the left ventricle[15]. Proper strategies to support the right ventricle are of major importance. In addition, Impella RP or RD may be one of them. In this article, we aim to review effectivity, physiological effects and outcome of temporary mechanical right ventricular support in patients with right ventricular failure.

Methods

Search strategy

We conducted a systematic review of studies that report about the effect of Impella RP or RD on right ventricular failure. We searched the PubMed Database on December 23th, 2018, using the following search strategy: ((“Ventricular Dysfunction, Right”[Mesh] OR “Right Ventricular Dysfunction”[TIAB] OR “right ventricular failure”[TIAB]) OR (“Hypertension, Pulmonary”[Mesh:noexp] OR “pulmonary hypertension”[TIAB]) OR ((“heart failure”[MeSH Terms] OR heart failure[TIAB]) AND right[TIAB]) OR “right heart failure”[TIAB]) AND impella[TIAB] AND English[lang].

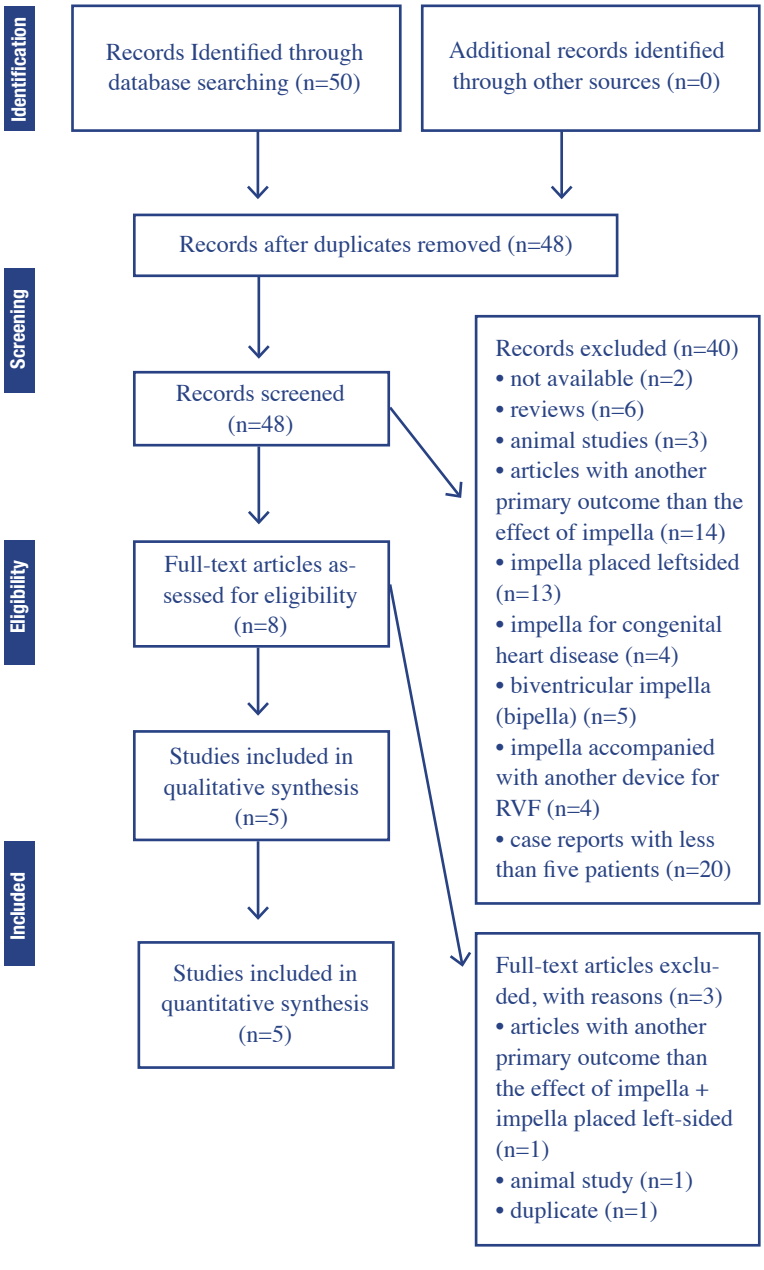
Inclusion and exclusion criteria

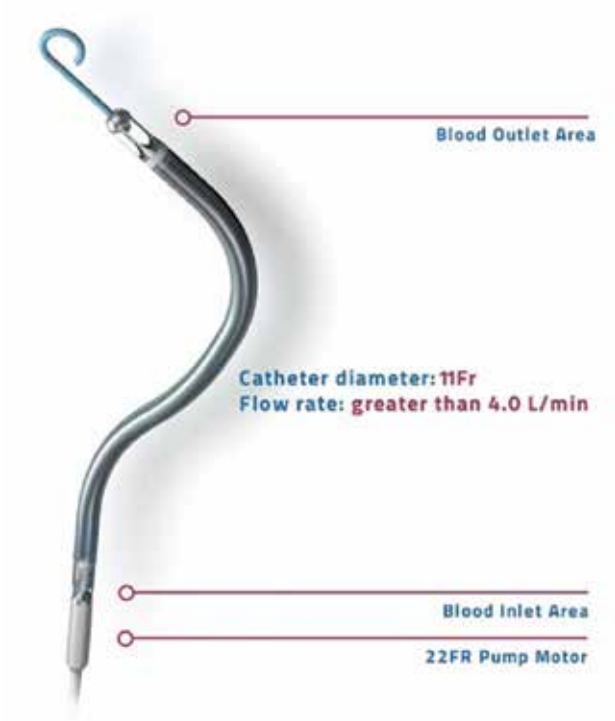
Inclusion was based on English articles, with 5 or more included

patients, describing the effect of a right sided Impella when right heart dysfunction was present.
The exclusion criteria were: no full text available, reviews, animal studies or duplicates, another primary outcome than the effect of Impella, left-sided or biventricular Impella (Bipella), Impella combined with another device for RVF and case reports with less than five patient reports. We screened the articles on title and abstract (Appendix).

Quality-assessment
We assessed the quality of the studies by using an adjusted version of the Newcastle Ottawa Scale (NOS) for cohort studies [17] (Appendix). We decided to use a 30-day follow up as long enough for outcomes to occur. For each item included in the article, we assigned a star, with a maximum of 6 stars. Articles

Figure 1 - Flowchart





with more than four stars were seen as articles with good quality. For case series we used the Process checklist [18] (Appendix), which we modified into a quality-assessment tool. For each item included in the article, we assigned a star, with a maximum of 29. We stated articles with less than 15 stars as methodologically weak. We independently selected the articles, disagreements were discussed and resolved by consensus.

Statistical analysis
Some articles contained information about more than one device, in which we only used the results about Impella RD or RP. Thus, we left out the results about Impella LD/LP systems. We converted the 30-days mortality into 30-days survival, to improve comparability. Also, we calculated the improvement (%) for the increase in cardiac index and the decrease in Central Venous Pressure ourselves. When the average age was not available, we calculated the average. At last we calculated the percentages of occurrence of each complication per study.

Results

Search Strategy
Our PubMed search resulted in 50 articles. We screened on title and abstract, whereafter we excluded 42 articles. After reading the full-text articles, we excluded three more articles. One of the articles was a duplicate, one was an animal study and the last one did not have the effect of Impella on RVF as primary outcome. Eventually we included five articles, two case series and three cohort studies. The flowchart in Figure 1 shows how we selected our articles. We excluded some articles on multiple criteria (Appendix).

Quality Assessment
Table 1 and 2 show the results of the quality assessment of the NOS[17] and the PROCESS checklist[18].

Study characteristics (Table 3)

We summarized the characteristics of the five included studies in Table 3.The numbers of patients included in the studies varied between 5 and 30. The mean age of the patients was between 50 and 60 years in all five studies. The study population differed between the five studies, but the endpoint was the same, namely the effect of Impella on RVF. Two types of Impella (Impella RD and RP) were used. Two of the five studies only used the Impella RP, another two studies only used the Impella RD and one article used both Impella RP and RD. The mean duration of device support varied between 3.04 and 7 days.

Study results

Cardiac Index (Table 4)

The effect on the Cardiac Index (CI) was reported in three of the five studies. In Elder et al.[19] an increase in CI of 47,9% (1,69L/min/m2 vs 2,50 L/min/m2) was observed, in Anderson et al.[20] an increase in CI of 81,3% (1,82 L/min/m2 vs 3,3 L/min/m2; p<0,001) was observed and in the study of Cheung et al.[3] an increase in CI of 23,8% (2,1 L/min/m2 vs 2,6 L/min/m2; p=0,04) was observed.

Central Venous Pressure (CVP) (Table 4)

The decrease in CVP was reported in four of the five studies. Elder et al.[19], Anderson et al.[20], Cheung et al.[3] and Sugiki et al.[12] all described a decrease in CVP of respectively 29,2% (24 vs 17 mmHg), 34,4% (19,2 vs 12,6 mmHg; p<0,0001), 31,8% (22 vs 15 mmHg; p<0,01) and 39,2% (15,3 vs 9,3 mmHg; p=0,005).

Table 1 - NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE COHORT STUDIES [17]

Author	Selection	Comparability	Outcome	Stars (total)
Cheung et al.[3] (2014)	1.a* 2.- 3.a* 4.a*	1.-	1.b* 2.a* 3.a*	6*
Anderson et al.[20] (2015)	1.a* 2.- 3.a* 4.a*	1.-	1.b* 2.a* 3.a*	6*
Granfeldt et al.[21] (2009)	1.a* 2.- 3.a* 4.a*	1.-	1.b* 2.a* 3.a*	6*

Survival at 30 days (Table 4)

The survival at 30 days was reported in four of the five articles. The lowest survival rate was described in the study of Granfeldt et al.[21], in which they only used the Impella RD: only 25% (two of the eight patients) was alive after 30 days [21]. Other survival rates were 72% in the study of Cheung et al.[3], a study in which 15 patients received the Impella RD and 3 patients the Impella RP. At 30 days 73,3 % of the patients in the study of Anderson et al.[20] was alive and in Elder et al.[19], 100% of the patients was alive after 30 days. These last two studies both only included patients that received the Impella RP.

Survival to discharge (Table 4)

Survival to discharge was reported in three of the five studies. In Elder et al.[19] a survival to discharge of 100% was observed. In the article of Anderson et al.[20] showed a slightly lower rate, which was 73,3%. The survival to discharge in the study of Sugiki et al.[12] was 14%.

Table 2 - PROCESS Checklist for included Case series [18]

Author	Title	Abstract	Introduction	Methods	Results	Discussions	Conclusions	Additional information	Stars (total)
Elder et al.[19] (2018)	1.-	2a.* 2b.* 2c.* 2d.*	3.*	4a.- 4b.- 4c.* 4d.* 4e.* 4f.* 4g.* 4h.- 4i.- 4j.*	5a.* 5b.- 5c.* 5d.* 5e.*	6a.* 6b.* 6c.* 6d.*	7a.* 7b.-	8a.- 8b.-	20*
Sugiki et al.[12] (2009)	1.-	2a.* 2b.* 2c.* 2d.*	3.*	4a.- 4b.- 4c.- 4d.* 4e.* 4f.* 4g.* 4h.- 4i.- 4j.*	5a.* 5b.- 5c.* 5d.* 5e.*	6a.* 6b.* 6c.- 6d.*	7a.* 7b.*	8a.- 8b.-	19*

Table 3 - Study characteristics

Author	Country	Study design	N	Mean age (years)	Study population	Impella type used	Total duration of device support (days)
Elder et al.[19] (2018)	Detroit, United States	Case series	5	50,8* (range: 28-72)	Shock due to massive or submassive Pulmonary Embolism	Impella RP	3.2 days (range of 1-6 days)
Anderson et al.[20] (2015)	United States	Cohort study	30	59 ± 15 (range: 24-86)	RVF after LVAD implantation or RVF after cardiotomy or RVF after myocardial infarction	Impella RP	3.04 ±1.5 days (range 0.5-7.8 days)
Cheung et al.[3] (2014)	Canada	Cohort study	18	57±10 (range N.A.)	RVF after acute myocardial infarction, post-cardiotomy, post-transplant, post-LVAD and myocarditis	Impella RD (15) and Impella RP (3)	7 days (range 2 - 19 days)
Granfeldt et al. [21] (2009)	Sweden	Cohort study	8	55,8 (range 26-84)	All Impella RD were used for acute right ventricular failure when weaning from CPB	Impella RD	4.1 days (range, 0,1 - 9 days)
Sugiki et al. [12] (2009)	Germany	Case series	7	54±7 (range: 45-62)	Temporary support after heart transplantation, after repeat mitral valve replacement, with a LVAD	Impella RD	4.9 ± 4.5 days (range 1-13 days)

NA= Not Available

**Self-calculated value*

Table 4 - Physiological effects and outcome of Impella RP and RD

Author	Effect on the Cardiac Index (L/min/m2) (%)	Effect on the Central Venous Pressure (mmHg) (%)	Survival at 30 Days (%)	Survival to discharge (%)
Elder et al. [19] (2018)	1,69 L/min/m2 vs 2,50 L/min/m2 increase of 47,9%*	24 mmHg vs 17 mmHg** (NA****) Reduction of 29,2%*	100% (5 of 5)	100% (5 of 5)
Anderson et al. [20] (2015)	1,82±0,04 vs 3,3 ± 0,23 liters/min/m2 (p= <0,001) increase of 81,3%*	19,2 ±0,7 vs 12,6 ±1 mmHg (p=<0,0001) Reduction of 34,4%*	73,3% (22 of 30)	73,3% (22 of 30)
Cheung et al. [3] (2014)	2,1 ±0,5 vs 2,6 ±0,6 liters/min/m2 (p=0,04) increase of 23,8%*	22±5 vs 15±4 mmHg (p=<0,01) Reduction of 31,8%*	72% (13 of 18)	NA
Granfeldt et al. [21] (2009)	NA	NA	25% (2 of 8) ***	NA
Sugiki et al. [12] (2009)	NA	15,3±1,4 vs 9,3±1,2 (p=0,005) Reduction of 39,2% *	NA	14% (1 of 7)

NA = Not Available

**Self-calculated value*

*** The CVP after treatment with Impella was only mentioned in two of the five patients. Therefore, the average is based on only those two patients. We calculated the values ourselves.*

****We converted the 30-days mortality to 30-days survival, so the results are more comparable.*

*****In the study of Elder et al, the p-value was not available.*

Table 5 - The complications of Impella RD and RP implantation

Author	Overall bleeding rate (%)	Escalation to a surgical RVAD needed (%)**	Hemolysis on support (%)	Rates of pulmonary embolism (%)	Recurrence of RV failure (%)	Death (%)%
Elder et al. [19] (2018)	NA	NA	NA	NA*** 0/30 patients	NA	0,0%*
Anderson et al. [20] (2015)	60%	3/30 patients 10,0%*	13,3%*	0,0%*	NA	8/30 patients 26,7%*
Cheung et al. [3] (2014)	NA	NA	2/15 patients with Impella RD 13,3%* 0/3 patients with Impella RP 0,0%*	NA	5/9 patients 55,6%*	9/18 patients 50,0%*
Granfeldt et al. [21] (2009)	NA	NA	NA	NA	NA	7/8 patients 87,5%*
Sugiki et al. [12] (2009)	2/7 patients 28,6%*	NA	NA	0/7 patients 0,0%*	1/7 patients 14,5%*	6/7 patients 85,7%*

NA= not available

**Self-calculated value*

***Only suitable for Impella RP.*

****Patients included for this study were patients who suffered from (massive) pulmonary embolism, so there are no data of PE as a complication of the impella RP.*

Complications of Impella implantation (Table 5)

In all five articles complications of Impella were described. In two of the five studies the overall bleeding, all cases of bleeding, was described. In the article of Anderson et al.[20] overall bleeding occured in 60% of the cases and in Sugiki et al.[12] in 28,6% of the cases. Only Anderson et al.[20] reported data about the need for implantation of a surgical RVAD when the RV did not recover well enough with the Impella RP device. An RVAD is a RV-assist-device, which allows the recovery of RV-function[22]. The surgical RVAD was eventually placed in 3 out of 30 patients (10,0%).

Hemolysis was reported in two of the five studies. In Anderson et al.[20] they reported hemolysis on support in 13,3% of the cases. In Cheung et al.[3] hemolysis on support occured in 13,3% of the cases with Impella RD and in 0% of the cases with Impella RP. In the study of Anderson et al.[20] and Sugiki et al.[12] a 0% rate of pulmonary embolisms in their cohorts was reported.

The rate of recurrence of RV failure was documented in two of the five studies. In Cheung et al.[3] 55,6% of the patients had recurrence of RVF one year after explantation of the Impella RD or RP. In Sugiki et al.[12] 14,5% of the patients had recurrence of RV failure after explantation of the Impella RD, of whom 100% died. All articles reported mortality rates. In the study of Elder et al.[19] they do not describe any deaths (0,0%), which is in contrast with the other studies. In Sugiki et al.[12] and Granfeldt et al.[21] they described a great mortality rate, respectively 85,7% and 87,5% of the patients died. In the article of Sugiki et al.[12], pumpstop occurred in two cases, which led to circulatory arrest in one patient. The study of Anderson et al.[20] reported a lower mortality rate of 26,7% and Cheung et al.[3] described a mortality rate of 50%.

Discussion

In this systematic review we investigated the effectivity, physiological effects and outcome of temporary mechanical right ventricular support (Impella) in patients with right ventricular failure. The results in the literature support the hypothesis that an Impella, a temporary mechanical RV support, leads to CVP decrease, and CI increase, thus unloading the RV[3,12,19-21]. This suggests that temporary support can unload the right ventricle in order to facilitate recovery of right ventricular failure.

The most common complications of the implantation of Impella RP and RD were overall bleeding, escalation to a surgical RVAD, hemolysis on support, recurrence of RV failure and death[3,12,19-21].

The mortality rate was higher in the studies of Granfeldt et al.[21], Cheung et al.[3] and Sugiki et al.[12]. These studies mainly used the Impella RD. In the studies of Elder et al.[19] and Anderson et al.[20] the Impella RP was used and the mortality rate was lower. This may suggest that Impella RP results in less adverse outcomes than Impella RD. The difference between the Impella RP and RD is that the Impella RP can be placed percutaneously, while the Impella RD is implanted through a sternotomy. Maybe the higher mortality in the Impella RD group is a consequence of the more complex surgery.

This could lead to underestemation of the results of Impella RP. The small amound of available data about the Impella RD and the Impella RP did not make it possible to look at these two devices separately. When more literature is available about the Impella device, it can be very informative to look at the effects of the Impella RD and Impella RP separately.

Furthermore, we think that the safety and mechanical reliability of the Impella RD system should be further investigated. In the article of Sugiki et al.[12] pumpstop occurred in two cases, which led to circulatory arrest in one patient. This is a very serious adverse event, which is why we think that the Impella RD should not be used clinically until its mechanical reliability is improved.

The limited available data on this new research area also made it impossible to perform a meta-analyse in our systematic review, because there was too much heterogeneity between the study populations of the articles. When the research area is more developed, a new systematic review could be done including a meta-analyse, which makes it easier to compare the articles.

We also think it is important to point out that the articles we used were a combination of cohort studies and case reports. These studies are not as comparable as we would like to, but because of the few available studies, we had to use both. This may have caused different outcomes and should be kept in mind when reading this article.

After we made our final search strategy, a new article was published on Pubmed. This article contained more patients than the other articles we used and could have been useful if it would have been published before we made our final search strategy. [23]

Furthermore, we could not be strict on the selection of the available articles due to the limited data. Hereby, we included all articles that contained data about the effect of Impella on RVF. We were not able to select on the reason for placement of the Impella RD or RP, which could be of great importance for the outcomes. Further research is needed in order to support the evidence of this feasible mechanical option in patients with right ventricular failure.

References

1. *Kaul T, Fields B. Postoperative acute refractory right ventricular failure: incidence, pathogenesis, management and prognosis. Cardiovasc Surg 2000;8:1-9.*
2. *Haddad F, Couture P, Tousignant C, Denault A. The right ventricle in cardiac surgery, a perioperative perspective: II. Pathophysiology, clinical importance, and management. Anesth Analg 2009;108(2):422-433.*
3. *Cheung A, White C, Davis M, Freed D. Short-term mechanical circulatory support for recovery from acute right ventricular failure: clinical outcomes. J Heart Lung Transplant 2014 August;33(8):794-799.*
4. *Kapur N, Paruchuri V, Korabathina R, Al-Mohammdi R, Mudd J, Prutkin J, et al. Effects of a percutaneous mechanical circulatory support device for medically refractory right ventricular failure. J Heart Lung Transplant 2011 December;30(12):1360-1367.*
5. *Pande S, Tewari P, Agarwal S, Agarwal V, Agrawal V, Chagtoo M, et al. Evidence of apoptosis in right ventricular dysfunction in rheumatic mitral valve stenosis. Indian J Med Res 2016 November;144(5):718-724.*
6. *Mohammed S, Hussain I, AbouEzzeddine O, Takahama H, Kwon S, Forfia P, et al. Right ventricular function in heart failure with preserved ejection fraction: a community-based study. Circulation 2014 23 December;130(25):2310-2320.*
7. *Kerbaul F, Rondelet B, Motte S, Fesler P, Hubloue I, Ewalenko P, et al. Effects of norepinephrine and dobutamine on pressure load-induced right ventricular failure. Crit Care Med 2004 April;32(4):1035-1040.*
8. *LaCombe P, Lappin S editors. Physiology, Afterload Reduction. Treasure Island, Florida: StatPearls Publishing LLC; 2018.*
9. *Kapur N, Esposito M, Bader Y, Morine K, Kiernan M, Pham D, et al. Mechanical Circulatory Support Devices for Acute Right Ventricular Failure. Circulation 2017 July;136(3):314-326.*
10. *Ponikowski P, Voors A, Anker S, Bueno H, Cleland J, Coats A, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur J Heart Fail 2016 August;18(8):891-975.*
11. *Calafiore A, Iacò A, Romeo A, Scandura S, Meduri R, Varone E, et al. Echocardiographic-based treatment of functional tricuspid regurgitation. J Thorac Cardiovasc Surg 2011 August;142(2):308-313.*
12. *Sugiki H, Nakashima K, Vermes E, Loisance D, Kirsch M. Temporary right ventricular support with Impella Recover RD axial flow pump. Asian Cardiovasc Thorac Ann 2009 August;17(4):395-400.*
13. *Danton M, Byrne J, Flores K, Hsin M, Martin J, Laurence R, et al. Modified Glenn connection for acutely ischemic right ventricular failure reverses secondary left ventricular dysfunction. J Thorac Cardiovasc Surg 2001 July;122(1):80-91.*
14. *Goldstein J, Barzilai B, Rosamond T, Eisenberg P, Jaffe A. Determinants of hemodynamic compromise with severe right ventricular infarction. Circulation 1990;82:359-368.*
15. *Peltan J, Oses P, Calderon J, Casassus F, Barandon L. Impella 5.0 microaxial pump as a right ventricular assist device after surgical treatment of posterior post-infarction ventricular septal defect. Perfusion 2014 September;29(5):472-476.*
16. *Impella RP®. 2019; Available at: http://www.abiomed.com/impella/impella-rp. Accessed September 25, 2019.*
17. *Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. 2018; Available at: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.*
18. *Agha R, Borrelli M, Farwana R, Koshy K, Fowler A, Orgill D, et al. The PROCESS 2018 statement: Updating Consensus Preferred Reporting Of CasE Series in Surgery (PROCESS) guidelines. Int J Surg 2018 December;60:279-282.*

19. *Elder M, Blank N, Kaki A, Alraies M, Grines C, Kajy M, et al. Mechanical circulatory support for acute right ventricular failure in the setting of pulmonary embolism. J Interv Cardiol 2018 August;31(4):518-524.*
20. *Anderson M, Goldstein J, Milano C, Morris L, Kormos R, Bhama J, et al. Benefits of a novel percutaneous ventricular assist device for right heart failure: The prospective RECOVER RIGHT study of the Impella RP device. J Heart Lung Transplant 2015 December;34(12):1549-1560.*
21. *Granfeldt H, Hellgren L, Dellgren G, Myrdal G, Wassberg E, Kjellman U, et al. Experience with the Impella recovery axial-flow system for acute heart failure at three cardiothoracic centers in Sweden. Scand Cardiovasc J 2009 August;43(4):233-239.*
22. *Sertic F, Ali A. Acute-right-ventricular-failure post-cardiotomy: RVAD as a bridge to a successful recovery J Surg Case Rep 2018;2018(6).*
23. *Khalid N, Rogers T, Shlofmitz E, Chen Y, Waksman R. Expanding the Treatment of Calcified Lesions. Cardiovasc Revasc Med 2019 July;20(7):622-623.*

Appendix

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE COHORT STUDIES [17]*

Selection

1) Representativeness of the exposed group/cohort.
   a) Truly representative of the average RVF patients in the community*.
   b) Somewhat representative of the average RVF patients in the community.
   c) Selected group of users.
   d) No description of the derivation of the group.
2) Selection of the non-exposed group/cohort.
   a) Drawn from the same community as the exposed group*.
   b) Drawn from a different source.
   c) No description of the derivation of the non-exposed group.
3) Ascertainment of exposure.
   a) Secure record*.
   c) Written self reports.
   d) No description.
4) Demonstration that outcome of interest was not present at start of study.
   a) Yes*.
   b) No.

Confounder

1) Comparability of groups on the basis of the design or analysis.
   a) Study controls for age and education*.
   b) Study controls for any additional factors*.

Outcome

1) Assessment of outcome.
   a) Independent blind assessment*.
   b) Record linkage.*
   c) Self reports.
   d) No description.
2) Was follow-up long enough (30 days) for outcomes to occur.
   a) Yes*.
   b) No.
3) Adequacy of follow up of cohorts.
   a) Complete follow up – all subjects accounted for*.
   b) Subjects lost to follow up unlikely to introduce bias*.
   c) Follow up < 70% and no description of those lost.
   d) No statement.

* For our systematic review we left out two questions of the Newcastle-Ottawa Quality Assessment Scale, namely: 'selection of the non-exposed group/cohort' of the Selection section and 'comparability of groups on the basis of the design or analysis' of the Confounder section.

PROCESS Checklist [18]

Section Item Checklist Description Page Number

Title

1) The words "case series" and the area of focus should appear in the title (e.g. disease, exposure/intervention or outcome).

Abstract

2a) Introduction - what is the unifying theme of the case series.
2b) Methods - describe what was done, how and when was it done and by whom.
2c) Results - what was found.
2d) Conclusion - what have we learned and what does it mean

Introduction

3) Explain the scientific background and rationale for the case series. What is the unifying theme - common disease, exposure, intervention and outcome, etc. Why is this study needed?

Methods

4a) Registration and ethics - state the research registry number in accordance with the declaration of Helsinki - "Every research study involving human subjects must be registered in a publicly accessible database" (this can be obtained from; ResearchRegistry.com or ClinicalTrials.gov or ISRCTN). State whether ethical approval was needed and if so, what the relevant judgement reference was?

4b) Study design - state the study is a case series and whether prospective or retrospective in design, whether single or multicentre and whether cases are consecutive or non-consecutive.

4c) Setting - describe the setting(s)and nature of the institution in which the patient was managed; academic, community or private practice setting? Location(s), and relevant dates, including periods of recruitment, exposure, follow-up, and data collection

4d) Participants - describe the relevant characteristics of the participants (comorbidities, tumour staging, smoking status, etc). Describe length and methods of follow-up.

4e) Pre-intervention considerations e.g. Patient optimisation: measures taken prior to surgery or other intervention e.g. treating hypothermia/hypovolaemia/hypotension in burns patients, ICU care for sepsis, dealing with anticoagulation/other medications and so on.

4f) Types of intervention(s) deployed and reasoning behind treatment offered (pharmacological, surgical, physiotherapy, psychological, preventive) and concurrent treatments (antibiotics, analgesia, anti-emetics, nil by mouth, VTE prophylaxis, etc). Medical devices should have manufacturer and model specifically mentioned.

4g) Peri-intervention considerations - administration of intervention (what, where, when and how was it done, including for surgery; anaesthesia, patient position, use of tourniquet and other relevant equipment, preparation used, sutures, devices, surgical stage (1 or 2 stage, etc). Pharmacological therapies should include formulation, dosage, strength, route and duration).

4h) Who performed the procedures - operator experience (position on the learning curve for the technique if established, specialisation and prior relevant training).

4i) Quality control - what measures were taken to reduce inter or intra-operator variation. What measures were taken to ensure quality and consistency in the delivery of the intervention e.g. independent observers, lymph node counts, etc

4j) Post-intervention considerations e.g. post-operative instructions and place of care. Important follow-up measures - diagnostic and other test results. Future surveillance requirements - e.g. imaging surveillance of endovascular aneurysm repair (EVAR) or clinical exam/ultrasound of regional lymph nodes for skin cancer.

Results

5a) Participants - reports numbers involved and their characteristics (comorbidities, tumour staging, smoking status, etc).

5b) Any changes in the interventions during the course of the case series (how has it evolved, been tinkered with, what learning occurred, etc) together with rationale and a diagram if appropriate. Degree of novelty for a surgical technique/device should be mentioned and a comment on learning curves should be made for new techniques/devices.

5c) Outcomes and follow-up - Clinician assessed and patientreported outcomes (when appropriate) should be stated with inclusion of the time periods at which assessed. Relevant photographs/radiological images should be provided e.g. 12 month follow-up.

5d) Where relevant - intervention adherence/compliance and tolerability (how was this assessed). Describe loss to follow-up (express as a percentage) and any explanations for it.

5e) Complications and adverse or unanticipated events. Described in detail and ideally categorised in accordance with the ClavienDindo Classification. How they were prevented, diagnosed and managed. Blood loss, operative time, wound complications, reexploration/revision surgery, 30-day post-op and long-term morbidity/mortality may need to be specified.

Discussion

6a) Summarise key results

6b) Discussion of the relevant literature, implications for clinical practice guidelines, how have the indications for a new technique/device been refined and how do outcomes compare with established therapies and the prevailing gold standard should one exist and any relevant hypothesis generation.

6c) Strengths and limitations of the study

6d) The rationale for any conclusions?

Conclusions

7a) State the key conclusions from the study

7b) State what needs to be done next, further research with what study design.

Additional Information

8a) State any conflicts of interest

8b) State any sources of funding

Reasons for exclusion

Exclusion criterion	Excluded articles
Not available	2.6
Reviews	4.19.30.31.38.43
Animal studies	24.37.40
Duplicates	32.33
Articles with another primary outcome than the effect of Impella	1.6.9.12.18.19.21.23.24.25.39.40.44.47
Impella placed left-sided	7.11.12.15.18.23.24.25.35.39.40.44.47
Impella for congenital heart disease	21.29.30.42
Biventricular Impella (Bipella)	10.11.13.17.49
Impella accompanied with another device for RVF	6.18.37.44
Case reports with less than 5 patients	1.5.6.10.11.14.16.17.18.20.21.22.23.27.35.36.41.42.44.48
Full-text articles excluded, with reasons	
- duplicate	3
- articles with another primary outcome than the effect of impella + impella placed left-sided	26
- animal study	50

The prevention of dangerous behavior through online challenges among young people

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Abstract

Objective: **Online challenges are a phenomenon with increasing popularity. Despite the innocence of most challenges, the consequences can be fatal. Because of this, it is relevant to educate children about the risks of dangerous online challenges (DOC) such as the choking challenge where self-strangulation is performed. The aim of this research is to investigate the possibilities for prevention of participation in DOC by young people.**

Methods: **Literature research on DOC and the possibilities for primary prevention was conducted. Furthermore, interviews with primary school students and (experience) experts and surveys among parents and teachers were carried out.**

Results: **Based on the literature, more than 75% of children are familiar with DOC and more than 10% has participated in a DOC. In literature regarding binge drinking, a similar dangerous youth phenomenon, theory of planned behavior (TPB) has shown to be important for the development of interventions to change health behavior. Furthermore, online-, school- and parent-based interventions have been shown to be effective in binge drinking. Therefore, these interventions may provide a useful basis in the primary prevention of DOC. A total of 34 students were interviewed, 215 teachers and 65 parents submitted an online survey. Interviewed students all participated in at least one online challenge and 85% of them informed their parents about this. The most effective preventative method, according to students and teachers (62% and 61% respectively), is education by an (experience) expert, which would be more effective than conventional educational methods.**

Conclusion: **Education by an (experience) expert concerning DOC and child health, combined with improved information provision for parents, could be used as a prevention measure against participation in DOC by young people.**

Introduction

On the 6th of May 2017 Tim Reynders, a 16-year-old boy from Arkel (the Netherlands), dies under suspicious circumstances showing signs of asphyxia.[1] One year later a similar case takes place in The Hague and a 15-year-old boy named Clay Haimé dies.[2] In both cases parents and local authorities first suspect suicide, but then a video of Tim performing the so-called "choking challenge" is found.

The choking challenge is only one of the many dangerous online challenges (DOC) that circulate on the internet. These online challenges have become more and more popular over the past years. The goal of such challenges is to perform a set task, videotaping the person performing the task and subsequently publishing the video on an online social media platform. These videos are then shared among contacts and motivate or even challenge others to do the same.[3] Online challenges are very diverse in execution as well as result, which immediately prompts an issue: not every online challenge is dangerous. The choking challenge performed by the aforementioned children can be categorized as a non-oxygenative challenge.[4] In total DOC can be divided into three categories: non-oxygenation challenges, dare challenges and aggression challenges.[5]

Non-oxygenation challenges

In this type of challenge the participant tries to prevent blood flow to the brain by compressing the carotid artery. This leads to oxygen deprivation of the brain and the buildup of carbon dioxide concentrations, which is experienced as a short-term 'high'.[4] However, if blood flow is restricted for too long, the oxygen deprivation may cause cerebral ischemia which can result in damage of brain tissue or even death. The risk of death increases as young people subsequently begin to carry out the challenge alone.[5] A survey among primary school students in France demonstrated that at least 19% of children aged 8 to 11 have participated in a non-oxygenation challenge.[3]

Dare challenges

This category concerns challenges in which an individual performs a certain task under group pressure. They can be relatively harmless, for example jumping into a ditch while it is freezing, but they can also be very dangerous. The Blue Whale Challenge (BWC) is an example of a life-threatening dare challenge. In this challenge, individuals must perform assignments imposed by an unknown 'master'. These assignments are initially quite innocent, but increasingly contain elements of self-mutilation and eventually suicide.[8]

Aggression challenges
During an aggression challenge, one party forces the other to perform a certain task. These challenges involve physical or psychological violence.[5]

The true prevalence of DOC is difficult to determine, because most cases do not end in hospital admission or death. On top of this, death due to the choking challenge may be confused with suicide and is therefore not always documented as the correct cause of death. A troubling trend is that children are participating in online challenges at an increasingly young age. A French study shows that 84% of interviewed children is familiar with the term DOC, and that 12% has participated in a DOC (5). According to Vinge, nearly 50% of children are familiar with DOCs and 19% of children between 8 and 11 have taken part in a non-oxygenative challenge.[3] Although the number of studies that have analyzed the prevalence of DOC is very limited, the numbers of children that participate in it call for action.

At this time the possibilities for prevention of participation in DOC by children and adolescents have not yet been studied to our knowledge. Due to the increasing prevalence of DOC and its potential lethal outcome, research into prevention is highly necessary. Therefore, our goal is to find an effective method of prevention against participation in DOC.

Methods

Literature review

What is the definition of a DOC and what is known about this phenomenon?
To find relevant literature, the following search query was used:

"E-gaming"[TiAb] OR ("challenge"[TiAb] AND "blue whale"[TiAb])

The term Blue Whale is used for a specific DOC where the challenged performs increasingly difficult tasks which end in self-harm. Articles found were screened based on title and abstract. Selected articles were then read in its entirety. Articles were excluded if they [1] did not cover DOC, [2] were a reaction to an earlier study or [3] were a case report. Publications that were not fully available through the Erasmus MC portal were also excluded.

What are the possibilities for primary prevention for DOC among children and young adults?
A limited amount of scientific literature is currently available on the subject of DOC prevention. However, one French article was found regarding the subject. Due to the limited amount of available literature on DOC, the link between binge drinking and DOC has been drawn. Prevention strategies for binge drinking have been widely researched. In addition, the intrinsic refractors in binge drinking are quite similar to those of DOC. Both deal with social interactions between young adults, such as peer pressure and the risk-taking nature of children. On top of this, both focus on addictive aspects. Non-oxygenative DOCs can become addictive due to the temporary 'high' that participants experience. Finally, in both cases, parents seem to have limited knowledge about their child's behavior, since both are performed without parents' approval and are conducted out of their sight.

To find relevant literature on binge drinking, the following search query was used:
("Risk-Taking/prevention and control"[Majr] OR "Binge Drinking/prevention and control"[Majr]) AND ("adolescent"[MeSH] OR "child"[MeSH] OR "young people"[TiAb])

Articles found were screened based on title and abstract. Selected articles were then read in its entirety. Articles that were [1] not a systematic review, [2] not focused on primary prevention of binge drinking, [3] suggested method proven not effective or [4] specific participants were excluded. Publications that were not fully available through the Erasmus MC portal were also excluded.

Fieldwork

Interviews with students
Interviews took place in Dutch elementary schools in grade 7 and 8. Prior to the one-on-one interviews, children were asked in groups if they had ever performed a DOC. Those that did were selected for an interview. In these interviews the knowledge of children and their suggestions for preventive measures regarding DOC were explored. Our goal was at minimum two school classes and a total of 25 willing participants. This with a preference for one elementary and one middle school class (the Dutch name for high school). However due to unwillingness to cooperate, especially middle schools, (further data can be found in the discussion section) only children from elementary school classes were interviewed. Parental consent for the interview was obtained through an informed consent form.

Survey among parents
An online survey was distributed to determine the extent of knowledge of parents about the topic of DOC and their suggestions for prevention strategies. This survey consisted of multiple-choice questions with an additional open space to answer the question, if the multiple-choice answers were not sufficient for the participant. The survey was aimed at parents with children of the age of 18 years and less. This difference between the survey and the interview to increase the number of survey participants. The online link was distributed to several Facebook groups. Survey results were analyzed quantitatively and qualitatively.

Survey among teachers
Another survey was targeted specifically at elementary and high school teachers. These surveys were distributed personally and were available in paper format as well as online. The link to the online survey was published on Facebook and emailed directly to the schools that were willing to participate in our study. Paper versions were collected one week after the drop off of the forms. The structure of the survey is comparable to that of the survey for parents.

Table 1 - Outcomes of interviews with students

Variable	Number and share of interviewed students (n=34)
Number of interviews conducted	34
Age of the students	10-12
Male	12
Female	22
Fulfillment of the challenge	
Performed a challenge (at least 1)	34 (100%)
Cinnamon challenge	7 (21%)
Location of the fulfillment of the challenge	
On the street	4 (12%)
On vacation	3 (9%)
At home/In friends' home	28 (82%)
Alone	6 (18%)
Response to parental authority	
Positive if parents would	28 (82%)
disapprove	
Negative if parents would	3 (9%)
disapprove	
Unsure	3 (9%)
Reported to parents prior to	29 (85%)
performing the challenge	
Prevention measures	
Provide information	29 (85%)
Information by an expert	21 (62%)
Information by a well-known	9 (26%)
person	
Information by a victim of DOC	9 (26%)
No permission from parents	15 (44%)

Interviews (experienced) experts

The interviews with experts were conducted in person as well as via telephone contact. The topics discussed include preventive measures, pediatric medicine and
internal motivation to participate in potentially dangerous activities. The interviewed experts were dr. van der Lely (pediatrician), dr. Oosterhof (pediatrician), dr. Bonekamp-Verbrugge (physician GGD) and dhr. Reynders (founder of stichting Tim). Experts were approached via email and selected based on their expertise. A semi-structured interview style was used.[7] Initially standard questions were asked in every interview, while further along in the interview, the questions were based on the response of the interviewee.

Data collection

For the one-on-one interviews with children, a semi-structured interview style was used.[7] Initially standard yes or no questions were asked in every interview, after which the interviewee was asked to elaborate. Their answers were documented and categorized. Later in the interview the interviewed were asked for their opinion on topics such as preventative measures, and again to elaborate this opinion (table 1). For the surveys data was collected via the online program and combined with the filled in paper version. The results of the multiple choice questions were then analyzed. Subsequently, the answers of the open style questions were first categorized and then the results analyzed.

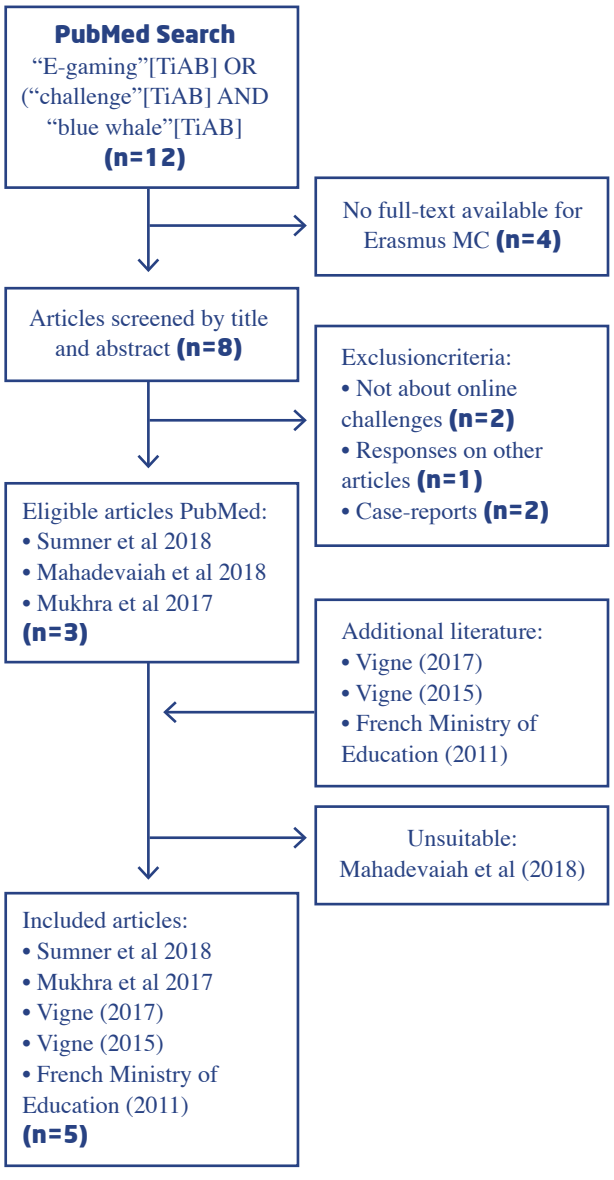
Results

Literature Review

What does a DOC entail and what is already known about this phenomenon?

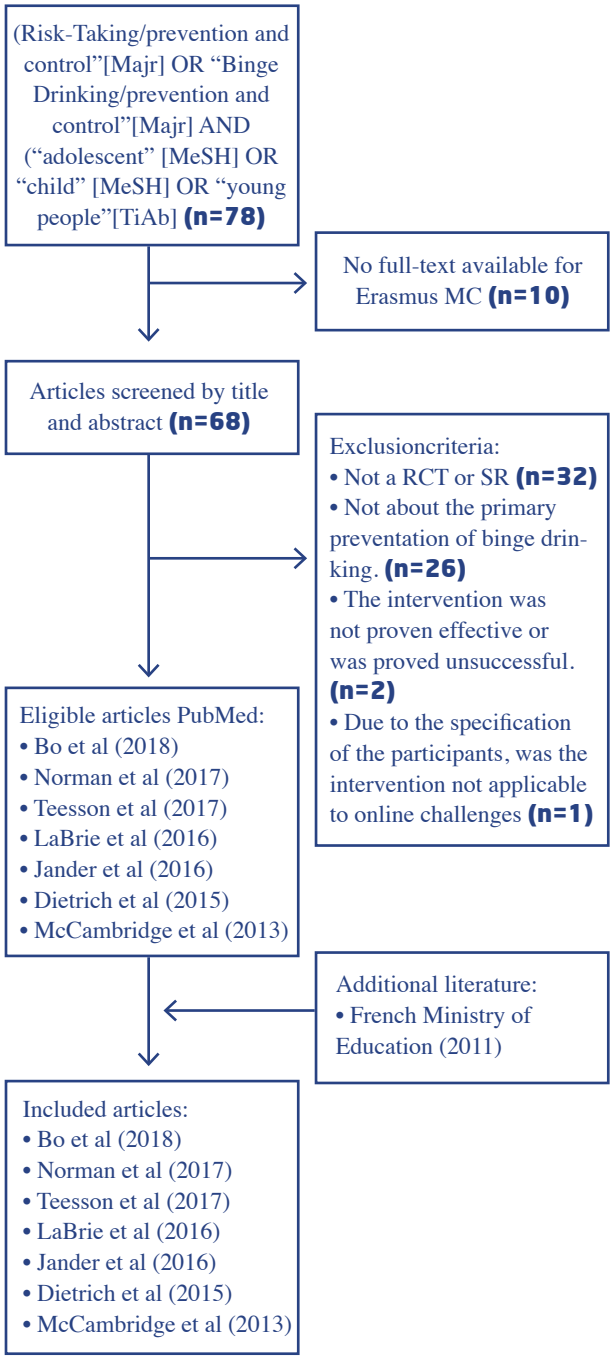
Our PubMed search yielded 12 articles. Four articles were not fully available through the Erasmus MC portal. After exclusion according to the criteria mentioned above, three articles were eligible, but one of these articles was later found inapplicable for our literature review. In addition, three French articles about DOC that have not been published on PubMed were found via the Jeudufoulard foundation. This organization collects literature on the subject of DOC. The three articles were translated and found to be eligible. Hence, a total of five articles was included in the final analysis (figure 1).

Figure 1- Flowchart of literature LOC



In France at least four in five children are familiar with DOC and more than 10% have participated in a DOC (5). Knowledge of the existence of DOC appears to occur more often in secondary school than in primary school (40% compared to 27%). Approximately 40% of the children who know what a non-oxygenation DOC entails have never discussed this experience with an adult. One in four children have witnessed a DOC. 92% of DOC appeared to have been performed on the school yard.[3] Another study by Vigne has confirmed the finding that DOC are mainly performed on school terrains.

Figure 2- Flowchart of literature primary prevention



What are the options for primary prevention of participation in DOC by young people?

Our PubMed search yielded 78 articles on this matter. Ten articles were not fully available through the Erasmus MC portal. After exclusion, seven items were found to be eligible. In addition, one French article that was used for the previous subquestion was also eligible for this sub-question. Accordingly, a total of eight articles was included (figure 2).

All prevention measures discussed in the included articles of PubMed are based on the theory of planned behavior (TPB). According to TPB, the intention is the most important determinant of behavior. The intention is determined by the attitude (pros and cons of behavior), the subjective norm (exemplary behavior, the opinion of others about the behavior and social support) and perceived behavioral control (assessment of confidence in the feasibility of healthy behavior).[8,9] TPB was found to be a strong theoretical framework for the development of interventions in order to change health-related behavior.[10]

Parent-based interventions

Parent-based interventions have been described to reduce binge drinking among young people.[11-13] Parenting behavior that has been determined to be related to alcohol use among young people includes general parenting strategies, alcohol-specific parenting strategies (including communication), parental drinking behavior and the relationship between parents and their children.[11]

Interventions that focus on alcohol-specific parenting strategies have smaller effect sizes compared to interventions that focus on both general and alcohol-specific parenting strategies.[11] An interactive feedback session, in which parents were motivated to adjust their alcohol-related communication by correcting misperceptions about alcohol, proved to be very effective. Predicted changes of binge drinking among students were found to be significantly lower after the intervention.[12,13]

School interventions

One study described school interventions as a preventative measure against binge drinking.[14] During the universal intervention (Climate), all students were given courses on alcohol and cannabis use at school. During the selective intervention (Preventure) only high-risk students participated twice in a group session. Long-term effects on binge-drinking were observed for the Climate-intervention alone, the Preventure-intervention alone and for a combination of both interventions. Combination of the two interventions proved to be no more effective than the Climate-intervention alone.[14] However, the Preventure method appears to be ineffective in the Netherlands.[15]

Online interventions

Most studies described online interventions to reduce binge drinking among young people .[10, 13, 16, 17]
A brief online intervention consisted of a combination of messages that focused on [1] the main TPB beliefs that underlie binge drinking, [2] self-affirmation manipulation to reduce defensive

Table 2 - Outcomes of the survey among parents

Variable	Number and share of completed surveys (n=65)
Number and age of the children	
Median number of children in the household	2
Average age of the children	13,8
Knowledge among parents	
Familiar with online challenges	41 (63%)
Knowledge about the participation of their children in a DOC	3 (5%)
Person in environment participated	15 (23%)
Familiar with a victim of DOC	4 (6%)
Of which deadly	3 (5%)
DOC discussed with own children	27 (42%)
Reasons for discussing	
Potential hazards	22 (34%)
An event related to DOC	3 (5%)
Other reasons	2 (3%)
Reasons for not discussing	
Unaware of existence of DOC	12 (18%)
Unaware of the hazards of DOC	4 (6%)
Thinks that own child will not participate in a challenge	12 (18%)
Other reasons	10 (15%)
Methods for prevention	
Make it discussable	36 (55%)
Provide information	16 (25%)

processing of the information and [3] implementation intentions (if-then plans to prevent binge drinking, so that positive intentions are translated into behavior). The TPB messages caused a significant reduction in the frequency of binge drinking. There were no significant effects on self-affirmation and implementation intentions.[10]

Two studies described a serious game with feedback as an online intervention. The focus of the feedback was to clarify general and personal consequences of alcohol consumption, in an attempt to change attitudes towards binge drinking. This intervention reduced binge drinking among young people.[13,16]

The timing of the interventions is important for their effectiveness. If the social and physical environment of the participants are in a state of change, it is easier to change certain beliefs. An example of such a period is the transition from high school to university.[10]

Prevention measures DOC

The guide on dangerous games and violent practices of the French Ministry of National Education recommends that the prevention of DOC should be part of a broader policy drafted by educational institutions, for the prevention of different types of risky behavior. The possible prevention measures must concern the entire community. The aim of prevention is to provide information about the existence of DOC, signs of participation and possible health risks. It is important that school principals warn other schools and institutions about DOC. Interventions should promote the self-respect of young people and the expression of emotions. According to the Ministry of National Education, various parties should offer support, such as doctors, school security, the Ministry of Internal Affairs and the National Federation for Victim support.[5]

Fieldwork

Interviews with students

Of the total 23 schools approached, 2 were cooperative in conducting interviews among the children. A total of 34 children -- 12 boys and 22 girls -- between the ages of 10 and 12 have been questioned using a semi-structured interview style (table 1).

Online challenges performed

All of the children had performed at least one (non-DOC) challenge, and 21% had performed the cinnamon challenge. In 82% of the cases the challenge was carried out in the home of the interviewee or in a friend's home. Moreover, 82% performed the challenge in the company of friends and/or family members, while the remaining 18% was alone. 82% of the interviewees noted that they would not have carried out the challenge in case their parents would have disapproved of the challenge in advance. Furthermore, 85% had informed their parents prior to performing the challenge. Luckily none of the interviewed had performed a true DOC, ignoring the debatable safety of the cinnamon challenge.

Possibilities for prevention

Of the interviewees who indicated that a school-based information session would be an effective preventive measure against participation in DOC (85%), 72% was convinced that this information session should be given by an expert or by an individual with personal experience with DOC. 27% of the interviewees proposed another preventive measure, namely spreading the stories of children who have suffered serious consequences or even death after performing a DOC. In addition, 44% of the interviewees believed that parents should not give their children permission to carry out a DOC.

Survey among parents

A total of 65 surveys have been completely filled in by parents (table 2). Of the participating parents, 37% was not familiar with the term 'online challenges' in general. 23% knew at least one person who had participated in a DOC, and within this group, 20% was aware of a death as a result of a DOC. Moreover, 42% of the parents indicated that they had discussed the topic DOC with their child(ren). De main reason for this was to discuss the potential dangers of DOC. Within the group of parents who had not discussed DOC with their child(ren), 18% specified that they did not believe that their child(ren) would participate in a DOC. Lastly, 55% of the parents responded that discussing the topic and making sure that talking about DOC is acceptable are important preventive measures.

Table 3 - Outcomes of the survey among teachers

Variable	Number and share of completed surveys (n=215)
Teachers	
Primary school teachers	95 (44%)
Secondary school teachers	120 (56%)
Median age students	11-15
Knowledge among teachers	
Familiar with online challenges	205 (95%)
Familiar with non-oxygenation challenges	78 (36%)
Familiar with a death due to DOC	12 (6%)
Familiar with injury due to non-oxygenation challenges	7 (3%)
Discussability of the topic and respective reasons	
Discussed with students	102 (47%)
Topical issue	37 (17%)
Safety of the students	24 (11%)
Alert role teacher	11 (5%)
Experience with DOC	7 (3%)
Routine	12 (6%)
Other reasons	11 (5%)
Motives of DOCs known to teachers	
Social pressure	108 (50%)
To act tough	54 (25%)
Curiosity	34 (16%)
Ignorance of the dangers	13 (6%)
Fear/uncertainty	6 (3%)
Prevention measures	
No prevention available	121 (56%)
Seen as task of the parents	27 (13%)
Information by an (experience) expert should be provided	130 (61%)
Adverse prevention measures	
Prohibition	120 (56%)
To provide too much attention to the problem	28 (13%)
Treated as topic not open for discussion	24 (11%)
Punishment	18 (8%)
To frighten about DOC	13 (6%)

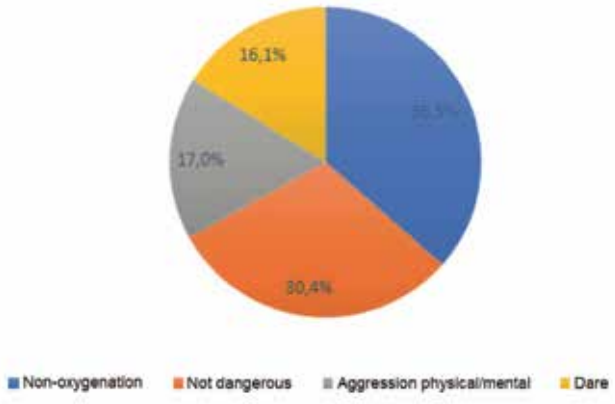
Survey among teachers

In this study, 215 surveys have been filled in by teachers (table 3); 95% of these teachers were familiar with the term 'online challenges', of which 37% were familiar with the term 'non-oxygenation challenges' (figure 3).

According to the responding teachers, the main motive to participate in DOC is social pressure (50%). 6% of the teachers were aware of a death as a result of a DOC. In cases of injury or death as a result of a DOC, 59% was the result of a non-oxygenation challenge. Almost half of the teachers (47%) had discussed the subject of DOC with students, mainly due to its topicality.

According to 56% of the responding teachers, not a single measure to prevent DOC was available through the school at which they work. 13% agreed that prevention is the responsibility of the parents. 61% of the teachers noted that information sessions given by an expert or by an individual with personal experience with DOC would be the most effective preventive measure (figure 5).

Figure 3 - Most mentioned online challenges by teachers



According to the survey responses, counterproductive preventive measures would be prohibition of DOC (56%) and making discussion about the topic a taboo (11%).

Interviews with experts

Three medical professionals (dr. van der Lely, dr. Oosterhof, dr. Bonekamp-Verbrugge) and one expert on the basis of personal experience with DOC (Mr. Reynders) have been interviewed in this study. All agreed that knowledge among adolescents about DOC should be improved. According to dr. van der Lely, dr. Bonekamp-Verbrugge and Mr. Reynders, parents are not sufficiently aware of the existence and the dangers of DOC. However, both dr. van der Lely and dr. Bonekamp-Verbrugge noted that parents should be the ones to inform their children about DOC and its dangers. On the contrary, dr. Oosterhof and Mr. Reynders mentioned that it should not be the task of parents to inform their children, but rather the task of health professionals, experts or an authority. In addition, dr. Bonekamp-Verbrugge and dr. van der Lely argued that the government also has a responsibility in controlling participation in DOC, by providing information and enforcing regulations.

Furthermore, dr. Oosterhof indicated that there is currently an underestimation of the problem of DOC among physicians. She suggested that this underestimation is caused by a lack of education on online behavior in the training of physicians. Dr. Bonekamp-Verbrugge specified that further training for physicians on online problems among adolescents exists, but that it is mainly focused on the medical issues and not on the social aspects of DOC. In addition, this further training is non-compulsory and therefore, not all physicians choose to follow this training.

Both dr. Bonekamp-Verbrugge and Mr. Reynders mentioned that it is essential that schools do not avoid the topic of DOC, but work to make this topic a point of discussion. Mr. Reynders indicated that schools are unjustifiably apprehensive to discuss DOC with their students, in fear of potential adverse effects. However, providing information is essential to make adolescents aware of the risks of participation in DOC.

Lastly, dr. van der Lely suggested that an epidemiological analysis of the incidence of participation and injuries after DOC is necessary, prior to the development of preventive measures.

Approach of schools

A total of 23 schools have been contacted for participation in this study, of which 14 schools have been visited in person. Only two of these schools were willing to participate in the individual interviews with students. The remaining 21 schools did not agree to be part of our study. The 23 schools were selected at random, and after making contact it was discovered that three students who had attended these schools had died after participating in a DOC. These three schools in question refused to partake in this study, stating the delicacy of the topic to both students and teachers as the primary reason. Overall, four schools refused participation in this study due to the delicacy of the topic, while five other schools stated that they were too busy with ongoing studies and six schools did not reply after multiple attempts to contact them. Six schools agreed to distribute the survey for teachers among their employees.

Discussion

This is, to our knowledge, the first study in which prevention of DOC among young people is studied. The results of this analysis show that education by an (experience) expert concerning DOC and child health, combined with improved information provision for parents, could contribute to prevent the participation of young people in DOC. This conclusion is supported by the literature about proven effective prevention measures against binge drinking among young people. In addition, the literature suggests that education should be supported with online interventions and parenting strategies.

The results from the interviews with primary school students and from the surveys among parents and teachers show that education by an (experience) expert is considered to be the most effective prevention measure. This is in line with the vision of some of the interviewed (experience) experts themselves. The education needs to emphasize the fact that participating in a DOC is very dangerous, especially when the DOC is performed by a person alone. Another conclusion is that parents are insufficiently familiar with DOC. The survey among parents shows that 37% of the parents has never heard of the term 'online challenges' in general. Moreover, a significant share of the parents that were aware of the phenomenon decided not to discuss it with their child(ren). Parents underestimate the chance that their child participates in a DOC and are generally unaware of the risks of DOC. However, our fieldwork, conducted based on interviews and surveys, demonstrates that a majority of the children has participated in at least one online challenge. The challenges were performed at home in 82% of the cases. In addition, 82% of the interviewed children stated they would listen to their parents if they would forbid them to participate in DOC. These results show that increasing the parents' knowledge and improving the communication between parents and their child(ren) about the topic are important bases for prevention measures. This could be accomplished through parent-based interventions.

In contrast to the limited knowledge of parents about DOC, teachers often appear to be well informed about the existence of this phenomenon. Some teachers stated that too little is being done to prevent participation in DOC. This shows that there indeed is support among teachers for the implementation of prevention measures. However, the generally rejective attitude of schools towards our request to conduct interviews among their students was striking (figure 6) and could be seen as a limitation of this study. Of the 23 approached schools, 41% indicated that they were not willing to participate in this study and 27% did not respond after multiple inquiries. None of the approached middle schools were willing to participate in an interview. The reasons stated to refuse participation in this study were similar to the issues the experts Mr. Reynders and dr. Oosterhof indicated, namely that the subject is too delicate. In addition, 55% of the schools stated that they did not prioritize this study due to a busy schedule. Three of the approached schools had experienced a death among their students as a result of participation in a DOC.

Figure 4: Reasons to discuss DOC with students

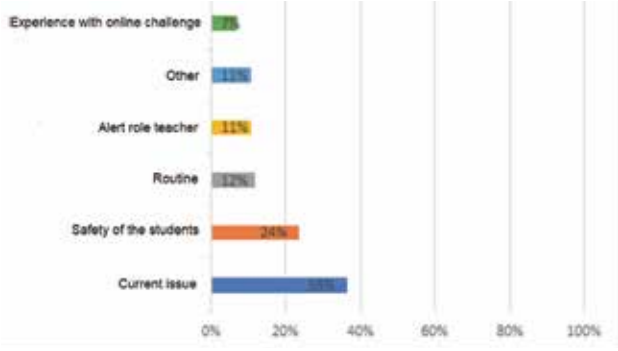


Figure 5: Effective prevention measures according to teachers

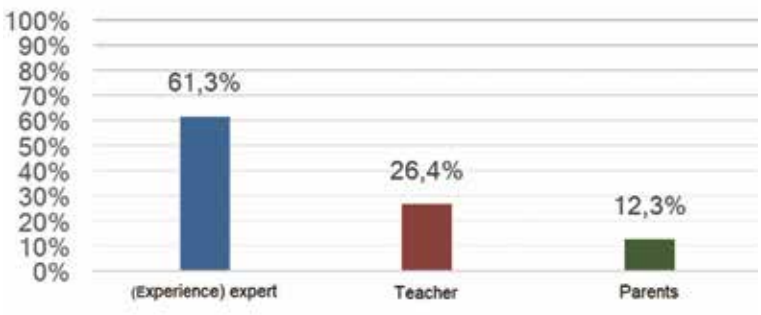
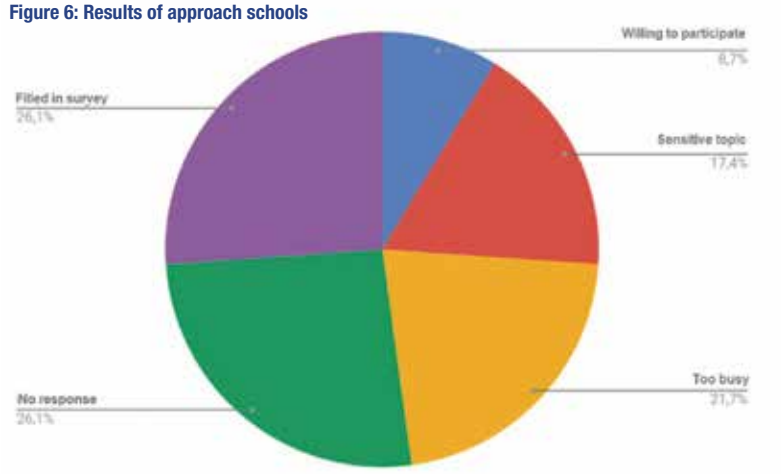


Figure 6: Results of approach schools



However, none of these schools were willing to participate in this study. A likely explanation of the high number of rejections received after approaching schools for this study, is that the management of these schools feared that bringing up the topic would only encourage students to participate in a DOC. However, as stated by Mr. Reynders, this is an unsubstantiated fear. It is important that schools are well informed about the expected positive effect of education about DOC.

Another limitation of this study is the small amount of available literature about DOC. Therefore, our literature review was primarily focused on the already extensively investigated prevention of binge drinking. These prevention measures, however, could form the basis for the prevention of DOC. Despite the fact that there are differences between these two subjects, such as in terms of age and level of awareness about the risks among parents and schools, there are also some important similarities: peer pressure, uninformed parents and health hazards apply to both subjects. These similarities may make it possible to use the prevention of binge drinking as a guideline for the development of prevention measures against DOC.

The literature research shows that the TPB offers a strong theoretical basis for the development of interventions to change behavior.[10] This theory can be used as a starting point for the development of prevention measures against DOC. Some of the prevention measures against binge drinking could clearly also be suitable for the prevention of DOC, such as parent-based interventions focused on parenting strategies and communication between parent and child on the topic. Short online interventions could also be successful, because these interventions have the potential to reach a large group of young people from different social classes and of different age groups. Lastly, school interventions such as educational courses about DOC should be developed. Participation in DOC happens more often in secondary school than in primary school.[3] Moreover, the timing of the interventions has proven to be important for their effectiveness.[10] During significant life transitions, the beliefs of young people are more amenable to change. It is therefore desirable to carry out school interventions during the period of transition from primary to secondary school.

References

1. *Stoffelen A. Nu ook in Nederland dode (16) bij extreme online 'challenge', ouders waarschuwen. Volkskrant. 2017; Available at: https://www.volkskrant.nl/nieuws-achtergrond/nu-ook-in-nederland-dode-16-bij-extreme-online-challenge-ouders-waarschuwen~bf2267bb/. Accessed 01/15, 2019.*
2. *de Goede A. Clay (15) overleden door 'onnozele challenge': 'Zijn verlies is afgrijselijk'. RTL nieuws. 2018; Available at: https://www.rtlnieuws.nl/nieuws/nederland/artikel/4200111/clay-15-overleden-door-onnozele-challenge-zijn-verlies. Accessed 01/15, 2019.*
3. *Vigne M. "Dangerous Games" of primary school Keystage 2 pupils: an inventory in the service of a policy of awareness and prevention of the risks before secondary school. 2015:67.*
4. *Pardoen J. Informatie over de 'choking game' (het wurgspel). 2017; Available at: https://www.bureaujeugdenmedia.nl/informatie-choking-game-wurgspel/. Accessed 01/29, 2019.*
5. *Ministère de l'Éducation nationale. Jeux dangereux et pratiques violentes. 2010.*
6. *Mukhra R, Baryah N, Krishan K, et al.': A Game or Crime? Sci Eng Ethics 2019 Feb;25(1):285-291.*
7. *Baarda B, Esther B, Fisher T, et al. Basisboek kwalitatief onderzoek. 4th ed.: Noordhoff Uitgevers; 2018.*
8. *Mackenbach JP, Stronks K. 4.3 Gezondheidsbevordering. Volksgezondheid en gezondheidszorg. 6th ed.: Bohn Stafleu van loghum; 2016. p. 175.*
9. *Ajzen I. Attitudes, personality, and behavior.: Open University Press; 1988.*
10. *Norman P, Cameron D, Epton T, et al. A randomized controlled trial of a brief online intervention to reduce alcohol consumption in new university students: Combining self-affirmation, theory of planned behaviour messages, and implementation intentions. Br J Health Psychol 2018 Feb;23(1):108-127.*
11. *Bo A, Hai AH, Jaccard J. Parent-based interventions on adolescent alcohol use outcomes: A systematic review and meta-analysis. Drug Alcohol Depend 2018 Oct 1;191:98-109.*
12. *LaBrie JW, Earle AM, Boyle SC, et al. A parent-based intervention reduces heavy episodic drinking among first-year college students. Psychol Addict Behav 2016 Aug;30(5):523-535.*
13. *Jander A, Crutzen R, Mercken L, et al. Effects of a Web-Based Computer-Tailored Game to Reduce Binge Drinking Among Dutch Adolescents: A Cluster Randomized Controlled Trial. J Med Internet Res 2016 Feb 3;18(2):e29.*
14. *Teesson M, Newton NC, Slade T, et al. Combined universal and selective prevention for adolescent alcohol use: a cluster randomized controlled trial. Psychol Med 2017 Jul;47(10):1761-1770.*
15. *Lammers J, Goossens F, Conrod P, et al. Effectiveness of a selective intervention program targeting personality risk factors for alcohol misuse among young adolescents: results of a cluster randomized controlled trial. Addiction 2015 Jul;110(7):1101-1109.*
16. *Dietrich T, Rundle-Thiele S, Schuster L, et al. Differential segmentation responses to an alcohol social marketing program. Addict Behav 2015 Oct;49:68-77.*
17. *McCambridge J, Bendtsen M, Karlsson N, et al. Alcohol assessment and feedback by email for university students: main findings from a randomised controlled trial. Br J Psychiatry 2013 Nov;203(5):334-340.*

An uncommon cause of dysphagia

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Abstract

Acute pancreatitis is an acute inflammatory disease of the pancreas, which can be complicated by pseudocysts or walled-off pancreatic necrosis. Most pseudocysts are asymptomatic, but some may present with abdominal pain, weight loss and/or jaundice. If the pseudocyst is symptomatic, treatment is indicated. The European Society of Gastrointestinal Endoscopy (ESGE) guidelines recommend a step-up approach with percutaneous or endoscopic drainage as first treatment option.[1] Endoscopic drainage consists of transmural placed pigtail stent with one side in the fluid collection, and the other side in the lumen. The goal of this treatment is to facilitate a way to drain the fluid collection or pseudocyst into the lumen. Complications of this treatment can be stent occlusion, migration, recurrence of the fluid collection and secondary infection.[2] The ESGE recommends that these stents should be left long-term in-situ.[1]

Case presentation

A 84-year old male presented to the emergency department with nausea, vomiting and dysphagia since four days. He had a medical history of pancreatitis five years earlier which was complicated by recurrent fluid collections, for which he underwent trans-gastric drainage. Endoscopically placed pigtail stents were left indwelling since then.

On admission, patients vital signs were normal. Laboratory tests showed an elevated CRP of 74 mg/L, increased leukocyte count of 10.6 x 109/L, and a lipase of 84 U/L. Computed tomography (CT) of the abdomen demonstrated a small remaining peripancreatic fluid collection (figure 1), with the distal ends of two double pigtail plastic stents positioned in the collection. However, the proximal end of the plastic pigtail stents were located at the height of the lower esophageal sphincter (figure 2 and 3). Subsequently an upper gastrointestinal endoscopy was performed. The proximal ends of the plastic pigtail stents were seen in the lower part of the esophagus (figure 4), and the stents were removed by a grasping forceps. Immediately after removal of the stents the patient was free of symptoms and the day after could be discharged from the hospital. Further treatment was not indicated on the short term, as the patient was asymptomatic. However, three months after removal of the stents, the patient had to undergo an open marsupialization of the cyst. Informed consent was obtained after the surgery.

Figure 1: CT scan of fluid collection

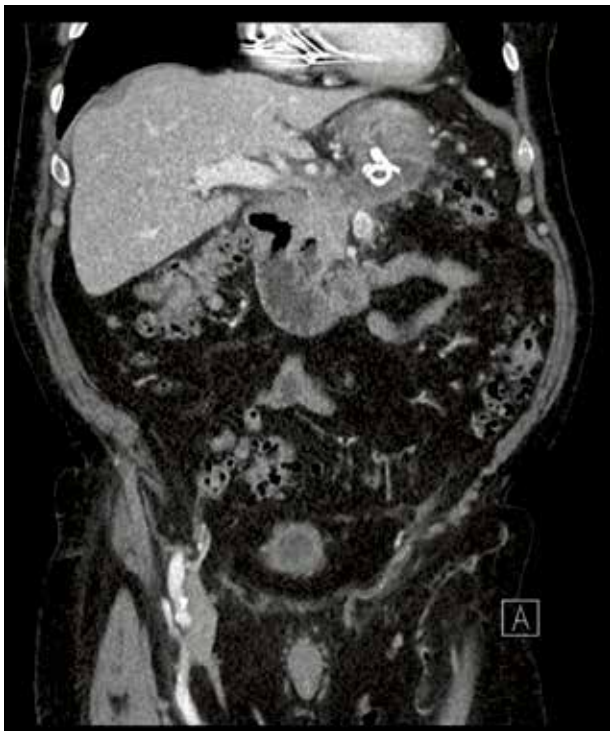
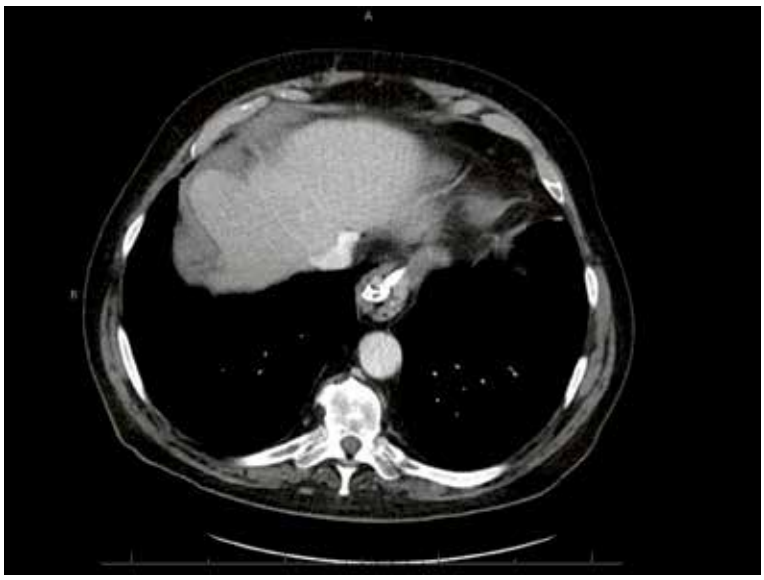


Figure 2: CT scan of stents in esophagus



Discussion

Although migration of pigtail stents occurs frequently (5.1-16.6%), this is the first case report to describe proximal stent migration to the esophagus causing dysphagia.[3,4] This case report shows that besides the more common causes of dysphagia migrated pigtail stents should be included in the differential diagnosis in patients with a history of endoscopically drained pancreatic fluid collections.

4. References

1. *Arvanitakis, M., et al., Endoscopic management of acute necrotizing pancreatitis: European Society of Gastrointestinal Endoscopy (ESGE) evidence-based multidisciplinary guidelines. Endoscopy, 2018. 50(5): p. 524-546.*
2. *Papachristou, G.I., et al., Peroral endoscopic drainage/debridement of walled-off pancreatic necrosis. Ann Surg, 2007. 245(6): p. 943-51.*
3. *Bazerbachi, F., et al., Metal stents versus plastic stents for the management of pancreatic walled-off necrosis: a systematic review and meta-analysis. Gastrointest Endosc, 2018. 87(1): p. 30-42 e15.*
4. *Rana, S.S., et al., Consequences of long term indwelling transmural stents in patients with walled off pancreatic necrosis & disconnected pancreatic duct syndrome. Pancreatology, 2013. 13(5): p. 486-90.*

Figure 3: CT-scan showing the double pigtail stent

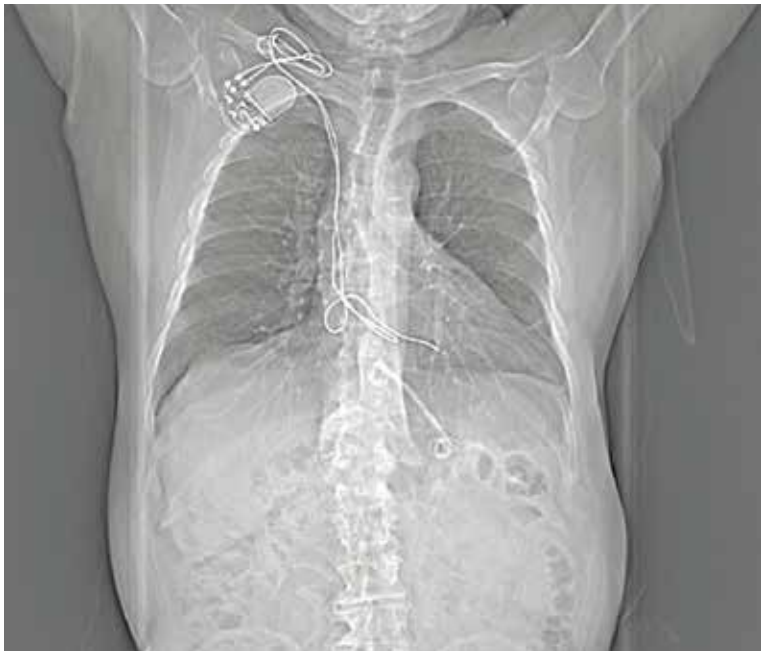
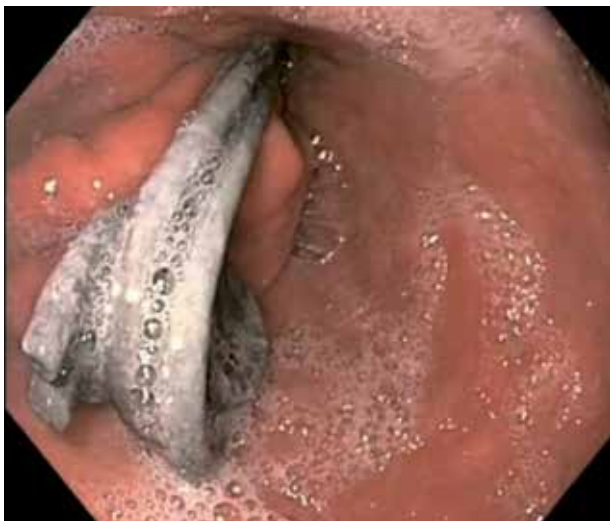


Figure 4: Image of gastroscopy showing the proximal end of the double pigtail in the lower part of the esophagus



Instructions for EJM authors

General

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

The section Authors instructions storyline, on the website (www.erasmusmc.nl/erasmusjournalofmedicine) will help you to organize your article in a logical, credible and readable way. This will help you - it tells you what goes where—and, thus, save you time. It will help the editors and peer reviewers—they will easily see the credibility and relevance of your work— and, thus, save them from writing rejection letters. And, it will help readers to quickly and easily read and understand your work and see its value.

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

Please be aware that we will have to return papers that do not conform to these instructions to the authors.

What you can enter

Research news - Research articles describe one study or analysis, usually from an elective research project or one of the masters programs. Number of words: max. 3500 + 4 figures or tables.

Extended abstracts - Extended abstracts consist of a condensed presentation of final or preliminary results of a study. Extended abstracts can concern ongoing research that is not yet published elsewhere which is comparable with a congress presentation thus does not require copyright transfer. An extended abstract can also be submitted after publication in another Journal if possible with extra figures, this does require proper referencing. Number of words: 350 words + 1 figure or table.

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

Systematic reviews - A systematic review is a literature review focused on a research question that tries to identify, appraise, select and synthesize all high quality research evidence relevant to that question in a quantitative way. Systematic reviews of high-quality randomized controlled trials are crucial to evidence-based medicine, and are considered very important by the editorial board of EJM. Besides health interventions, systematic reviews may concern clinical tests, public health interventions, social interventions, adverse effects, and economic evaluations. Number of words: 3000 + 3 figures or tables.

Opinion papers - These are papers that reflect the opinion of the author on a scientific topic. The author should be clear where evidence ends and personal opinion starts. A paper typically has a length of about 1000 words.

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

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

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

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

Comments - In this section editors, or faculty staff, as well students are invited to write a short critical comment on a paper, putting it into perspective for a broader medical public readership. Number of words: 350.

Letters to the editor - The editorial board encourages students to write a letter to the editor to comment on published papers, or on the journal in general. These will be published on the website of the journal. Letters should not exceed 200 words and may be abbreviated by the editor.

The review process

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

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

Instructions for EJM authors

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

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

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

Formatting instructions

Entry format - Papers should be submitted by email, to ejm@erasmusmc.nl. Word 2007 files are preferred for the initial submission. The file should include all figures and tables.

Title page - The title page should clearly identify the authors, the institute where the research project was carried out, as well as the staff member who supervised the project. The corresponding author name (first name and family name), email address, student id, should be clearly indicated. In case of multiple authors, state functions and departments only in superscript in alphabetical order.

Example:
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[a] Medical students, Erasmus MC University Medical Center Rotterdam, the Netherlands
[b] Dept. of Internal Medicine, Erasmus MC University Medical Center Rotterdam, the Netherlands
Correspondence: First name A.G. Family name, email: FirstnameFamilyname@me.com.

Structure - Please use the following sections in all papers (except in comments and opinion papers): Abstract, Introduction, Methods, Results, Discussion, References, Tables, Figures.

References - Number references in order of appearance. References should have the following format:
Rothwell, P. M. Medical and surgical management of symptomatic carotid stenosis. Int J Stroke. 2006; 1: 140-149. (I.e. year;vol:ppp-ppp) In case of more than 3 authors, name the first 3 and insert "et al.". Limit the number of references to 30. References should appear in the text as follows: "… treatment is of proven benefit.[1]"

Tables and figures - Tables and illustrations (both numbered in Arabic numerals) should be prepared on separate pages. Number tables and figures separately and consecutively. Tables require a heading and figures a legend, also prepared on a separate page and should be formatted with a text editor (example). Figures should be submitted electronically. B/w half-tone and color illustrations must have a final resolution of 300 dpi after scaling, line drawings one of 800-1,200 dpi (jpg and tiff is an acceptable format). Please note that all color-figures will be converted to gray tones. Please adapt graphs to suit this format, i.e. make use of dotted and dashed lines and hatched bars instead of colored items.. The final submission should contain figures as JPG or TIFF files.

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

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

Language
US English spelling and punctuation

The template for authors

Introduction

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

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

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

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

Methods section

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

What was studied and study design (subheading)

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

Data collection (subheading)

Describe the details of how the data was collected/observed

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

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

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

Data analysis (subheading)

Results section

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

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

Patient/animal characteristics
Data
Statistical results

Discussion section

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

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

Limitations (subheading)

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

Conclusions (subheading)

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

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

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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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Januari 2017, Editorial board of Erasmus Journal of Medicine.

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