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# RAMS

Radboud Annals of Medical Students

- Role of Adhesive Structures in Metastasis
- Revolution in Diagnosis of Pancreatic Cancer
- The Mysterious Piriformis Muscle Syndrome
- Neurotop: a Dive into the World of Neurology

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## COLOPHON


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Science invariably starts with a question. It can be posed by anyone inquisitive enough to ask and critical enough to think, irrespective of age and profession. As shown in the past, the best medical discoveries originate from unexpected answers, or better still, from answers to questions not yet asked. Take for example the serendipitous finding of penicillin. It was discovered because Alexander Fleming was careless enough to leave a dirty petri dish with bacteria in the lab sink when he went on vacation. Upon his return, he found out that the bacteria had not grown in an area where mould had formed.

Nevertheless, it is the quality of the question that is very - if not the most - important. In this edition of RAMS, the story of the Jack Andraka is told. At the age of fifteen, he asked himself how pancreatic cancer could be detected at an early stage. This burning question was fuelled by various experiences, of which the most important was the death of a close family friend due to pancreatic cancer. The solution to his question began to take shape whilst listening to his biology teacher in class and while he was concurrently reading a research article on nanotubes (read further on page #).

Although a great deal of knowledge about the aetiology and treatment of diseases has been generated over the past few decades, the current clinical practice is far from being completely evidence-based. This demands doctors to adopt a critical attitude towards the current clinical practise and a keen eye for improvement. For example, this edition contains a clinical lesson in which the authors advocate the consideration of the piriformis muscle syndrome as a possible diagnosis in patients with radiating back pain. Although case reports are often regarded as the lowest level of evidence, they add a relevant clinical observation and raise questions that warrant further scientific research. Importantly, case reports also provide unique patient-specific information that cannot be distilled from epidemiology, but is essential for doctors in their mission to procure patient-centered care. Here at RAMS, we strongly encourage students to help clinicians document these cases, as they often end up somewhere in a desk drawer.

The first research article in this edition is a perfect example of a narrative review; it discusses how cancer cells are able to metastasize and form new blood vessels. The authors have compiled the evidence that supports a role for specific structures involved in the adherence of cells to their environment during angiogenesis and metastasis. Based on this evidence, future research into targeted therapies aimed at preventing cancer cells from using adhesive structures as a means of spreading to other organs can be performed.

Furthermore, this edition contains an interview with prof. dr. Baziel van Engelen, neurologist at the Radboudumc. He talks about the Neurotop Summer School and the team of neurologists behind it. This extracurricular programme provides talented students with both an insight into the research field of Neurology and an opportunity to write their own research proposal. You can read more about this on page 16.

Assuming that the first sentence of this editorial holds true, I would like end by asking you this: what will your question be?

**Cas van der Made**
Editor-in-Chief

# INDEX

# THE INDISPENSABLE ROLE OF ADHESIVE STRUCTURES IN ANGIOGENESIS AND METASTASIS

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**ABSTRACT:**

**SUMMARY:** Adhesion of cells to the extracellular matrix is essential for a variety of physiological and pathological processes. Different adhesive structures have been described, such as focal adhesions, podosomes and invadopodia. All these structures exert their function through specific adhesion molecules, the integrins, and a variety of signalling molecules. Podosomes have been associated with the process of tumour angiogenesis. Furthermore, invadopodia are characteristic for invasive cancer cells and are linked to tumour invasion and metastasis. Cancer is one of the leading causes of death worldwide and the lack of a proper treatment is a growing problem. Angiogenesis and metastasis are major contributors to mortality in cancer patients. Besides the fact that angiogenesis stimulates tumour growth by increasing the supply of oxygen and nutrients, it also enables tumour cells to metastasize. Since adhesive structures have been associated with these processes, targeting components of adhesive structures could be an addition to the current cancer therapy.

**WHAT'S KNOWN**: Podosomes and invadopodia are cellular protrusions necessary in physiological and pathological conditions. Among these, angiogenesis and metastasis both contribute to the pathogenesis of cancer.

**WHAT'S NEW:** This review aims to summarise current knowledge on the role of podosomes and invadopodia in both angiogenesis and metastasis. Furthermore, novel prognostic markers in cancer therapy will be addressed and potentially curative therapies are discussed.

**KEYWORDS:** Podosomes, invadopodia, metastasis, angiogenesis, cancer treatment

**Abbreviations:** Arp2/3 complex: Actin related protein subunit 2 and 3 complex; ADAM proteases: a disintegrin and metalloproteinase; alpha-PIX: alpha-PAK-interacting-exchange factor; CDC42: cell division cycle protein 42 homolog; ECM: Extracellular matrix; ECs: Endothelial Cells; FAPα: fibroblast activating protein-α; GFP: Green Fluorescent Protein; HER2: Human epidermal growth factor receptor 2; MMPs: metalloproteinases; MT1-MMP: Membrane Type 1 Matrix Metalloproteinase; N-WASp: Neural WASp; PAK4: p21-associated kinase-4; PDGFRα Platelet Derived Growth Factor Receptor α; RGD: Arg-Gly-Asp; SNARE: soluble N-ethylmaleimide- sensitive factor-activating protein receptor; Src: proto-oncogene tyrosine-protein kinase Src Tks4: Tyrosine kinase substrate 4; Tks5: Tyrosine kinase substrate 5; VEGF: Vascular Endothelial Growth Factor; WASp: Wiskott-Aldrich Syndrome protein

## Introduction

Angiogenesis (the formation of new blood vessels) and metastasis are crucial players in mortality in cancer patients [1]. Angiogenesis contributes to the pathogenesis of cancer since it enables metastasis of tumour cells [2]. Cells assemble several structures to adhere to their environment. These adhesive structures have been associated with the processes of angiogenesis and metastasis. The adhesive structures podosomes and invadopodia have both been associated with pathological conditions [3,4]. In this review we describe the role of podosomes and invadopodia in physiological processes and cancer progression to ultimately identify novel prognostic markers and develop targeted therapies.

### Podosomes - the cellular feet - and their pathological counterparts: invadopodia

The cellular structures involved in the migration of cells and the degradation of the extracellular matrix (ECM) are called podosomes. Podosomes are ring-like structures that connect with the ECM via integrins which are known to provide a highly stabilised adhesion to the ECM, like feet on a surface, by reorganisation of the actin-cytoskeleton [5]. Podosomes contain a protrusive actin-rich core and are located at the ventral side of a polarized cell, enabling the cell to 'walk' over the ECM. They have been characterised in various cell types such as smooth muscle cells, osteoclasts, macrophages, dendritic cells and endothelial cells [3,5-7]. However, only podosome formation by endothelial cells are associated with angiogenesis promoting cancer progression.

Podosomes fulfil multiple functions in physiological processes. In contrast, invadopodia are protrusion-like structures that are selectively found in invasive tumour cells [4]. Though invadopodia are associated with pathological processes, no complete consensus regarding the similarities and differences of podosomes and invadopodia has been formalized.

In general, two different hypotheses have been described in previous studies as reviewed by Linder et al. [9]. Firstly, it has been suggested that podosomes and invadopodia are different structures and that cell types are not able to express invadopodia and podosomes simultaneously. This is supported by the fact that podosomes have been observed in endothelial cells, smooth muscle cells and monocytic cells, whereas invadopodia are mainly found in highly invasive cancer cells [8,10,11]. Other distinctive features of invadopodia compared to podosomes are the number and size of the adhesive structures, their lifetime and type of ECM degradation. The number of podosomes per cell is higher than invadopodia, namely 20-100 cell-1 compared to 1- 10 cell-1, respectively. Furthermore, invadopodia last up to one hour, whereas the lifetime of podosomes is approximately 2 to 12 minutes resulting in a high turnover rate of podosomes [12,13]. Lastly, while podosomes have a diameter of 0.5 - 1 μM, invadopodia show a diameter of about 8 μM [14]. This difference results from the fact that podosomes induce a relatively broad and superficial degradation of the ECM, whereas invadopodia induce a focused deep degradation enabling tumour invasion (Figure 1) [9,15].

The second theory is based on the argument that podosomes might differentiate into invadopodia, however the complete process has not been experimentally demonstrated yet [16,17]. For podosomes to become functional invadopodia, the size, the lifetime and the total num-

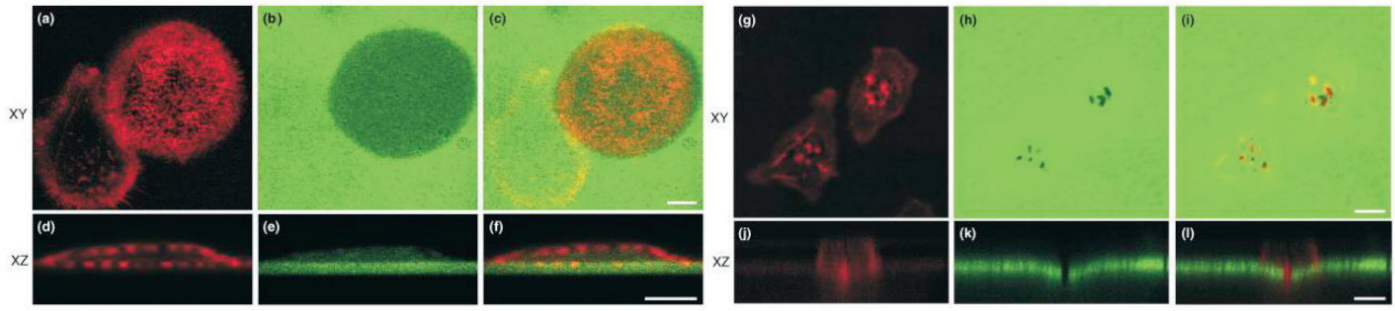


***Figure 1*** *Matrix degradation by podosomes in macrophages and invadopodia in carcinoma cells. (a-f) Primary human macrophages; (g-l) MTLn3 rat mammary adenocarcinoma cells seeded on Alexa488-labeled fibronectin (green) and stained for F-actin (red); (a,d,g,j) Red channel; (b,e,h,k) Green channel; (c,f,i,l) Merge. Matrix degradation results in a loss of colour, the perpendicular image ('XZ') demonstrates the depth of matrix degradation. Matrix degradation in macrophages is shallow and widespread caused by numerous podosomes. Matrix degradation by sarcoma cells is focalized and deeper caused by a few invadopodia. Figure adapted from Linder et al. [20].*

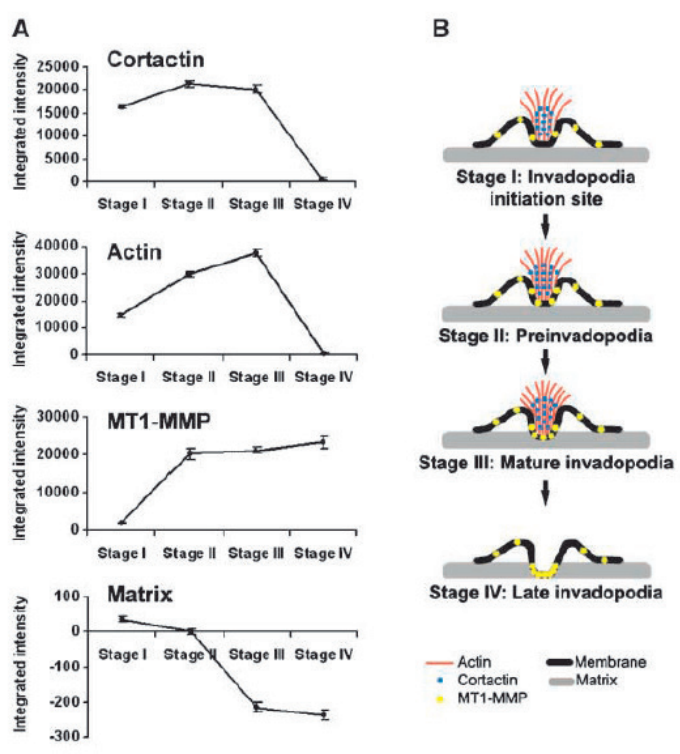


***Figure 2*** *Model of invadopodia formation and function. (A) Levels of cortactin, actin, and MT1-MMP at invadopodia are given and the degree of matrix degradation is quantified for each invadopodial formation stage. (B) Four stages of invadopodia formation and function are depicted. MT1-MMP: membrane type 1 matrix metalloproteinase. Figure adapted from Artym et al. [42].*

ber of the podosomes should be altered, as reviewed by Linder et al. [9]. Despite the lack of evidence of the actual transition of podosomes into invadopodia, certain characteristics have been described in previously performed studies. As previously mentioned, invadopodia have an increased lifetime compared to podosomes. Experiments using cofilin siRNA in invadopodia showed a decreased lifetime and less matrix degradation that more resembles characteristics of podosomes [18]. These individual processes were described in different experiments. However, it remains unknown whether all processes necessary for transition of podosomes into invadopodia can take place simultaneously. Although there are distinct differences between the two structures, Saltel et al. proposed the term 'invadosome' as an umbrella term for invadopodia and podosomes [19]. The presence of two different theories emphasizes the importance of investigating whether podosomes and invadopodia are different structures or invadopodia are being evolved from podosomes.

**Podosome and invadopodia formation**

Podosome formation can be initiated through activation of receptor tyrosine kinases by several growth factors. However, integrins are the main receptors responsible for the stimulation of podosome formation [20-22]. Several integrin subunits have been demonstrated to play a vital role in podosome formation [7,23,24].

Podosome formation is dependent on multiple pathways like the Phosphoinositide-3-kinase (PI3K) and Rho-Guanosine triphosphatase (Rho-GTPase) pathway [21,22]. Another downstream key signalling component in the formation of podosomes, is a specific Rho-GTPase called cell division cycle protein 42 homolog (CDC42) [25]. This signalling hub is essential for actin polymerisation and by activation of Wiskott-Aldrich Syndrome protein (WASp) it initiates actin branching [26].

Besides a wide number of structural proteins identified in podosomes, several matrix metalloproteinases (MMPs) have been identified in podosomes of a variety of cell types. Their function is inextricably linked to the ECM degrading capacity of podosomes. However, only the presence, recruitment and function of Membrane Type 1 Matrix Metalloproteinase (MT1-MMP) in podosomes has been thoroughly described. MT1-MMP has a major function in tumour angiogenesis which will be discussed further on.

The process of invadopodia formation depends greatly on similar processes as podosome formation, Artym et al. described this as a four stage process (Figure 2) [27]. Similar to podosome formation, invadopodia formation relies amongst others upon Arp2/3-mediated actin branching at the leading edge of the cell and therefore formation pathways are comparable [28]. Invadopodia formation is being explained in Figure 3.[10].

**Podosome involvement in tumour angiogenesis**

Angiogenesis plays a major role in metastasis since it facilitates intravasation of primary tumour cells. Angiogenic steps include endothelial cell activation, dissolution of the surrounding basement membrane by MMPs, increased endothelial cell proliferation and migration, tube formation, vessel anastomosis, and pruning to form a vascular network [29]. As mentioned before, endothelial cells possess podosomes that are enriched with MMPs and might therefore be involved in tumour angiogenesis [30].

The physiological processes involved in angiogenesis induce the release of several growth factors including Vascular Endothelial Growth Factor (VEGF) [31]. Recently, Seano et al. identified two distinguishable arrangements of podosomes present in endothelial cells stimulated by VEGF, namely individual podosomes and podosome rosettes at the basal side of the cells, which can be seen in Figure 4A [32]. Rosettes are ring-like structures in which podosomes are clustered together, aggregated by a

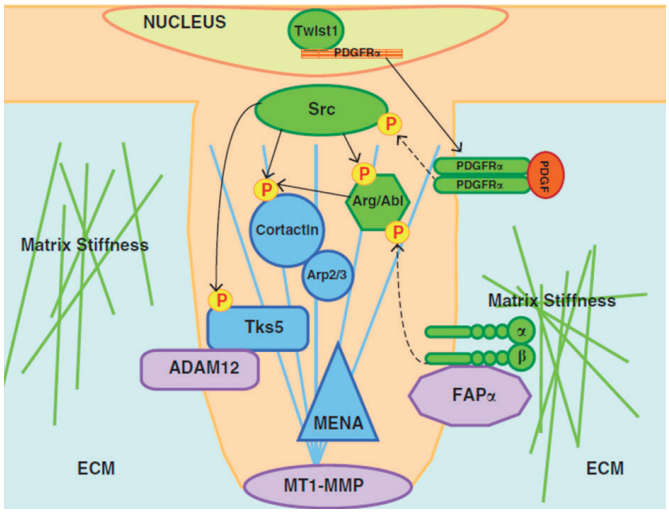


***Figure 3*** *Main components in invadopodia formation and function. Expression of PDGFRa, which is induced by Twist1, activates Src tyrosine kinase through phosphorylation. The activated Src tyrosine kinase induces invadopodia formation by phosphorylation of Tks5, cortactin and Arg/Abl . Invadopodia assembly triggers the recruitment of various proteases . PDGFRa: platelet derived growth factor receptor-α, Tks: tyrosine kinase substrate, Arg: Abl related gene. Regulatory components are indicated in green; blue indicates structural components of invadopodia which are important for invadopodia assembly (including the actin core); proteases are indicated in purple. Figure adapted from Paz et al. [10].*

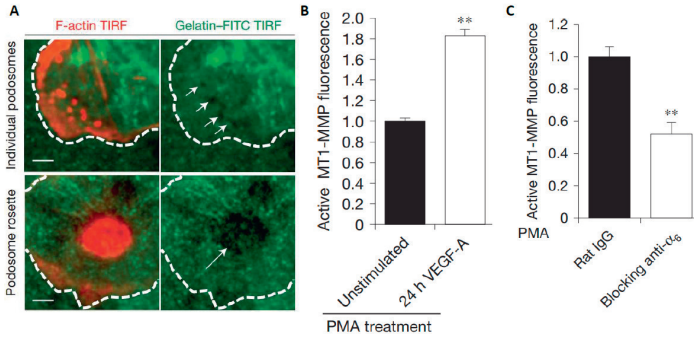


***Figure 4*** *Podosome and rosette formation stimulated by VEGF in endothelial cells and the inhibition of Membrane Type 1 Matrix Metalloproteinase (MT1-MMP) activity by α6 integrin antibody. (A) To determine proteolytic activity, endothelial cells were stained with phalloidin to visualize F-actin, and seeded on gelatin plates conjugated with FITC. Vascular Endothelial Growth factor-A (VEGF-A) was used to evoke an angiogenic response, and individual podosomes were compared to rosettes in terms of gelatin breakdown. The white dotted lines represent the cell boundaries, and the white arrows represent the decrease in fluorescence and gelatin breakdown. (B) Endothelial cells were treated for 30 minutes with the podosome stimulator phorbol-myristate-acetate (PMA). Angiogenic endothelial cells showed to possess 1.8 fold higher active MT1-MMP levels compared to quiescent endothelial cells, which was statistically significantly ($p<0.01$). (C) Endothelial cells treated with either Rat IgG or anti-α6β1 integrin antibody, followed by PMA treatment for 30 minutes. Addition of anti-α6ß1 integrin antibody significantly reduced MT1-MMP activation its gelatinolytic activity ($p<0.01$). In both B and C, normalized mean +/- SEM is depicted of three individual experiments using 9 x 104 cells. Statistical analysis in these experiments was performed using an unpaired non-parametric Mann-Whitney test. Figures adapted from Seano et al. [34].*

dense network of actin filaments [33]. Besides the induction of podosomes and podosome rosettes in endothelial cells, Seano et al. demonstrated that the activity of MT1-MMP was significantly increased in angiogenic endothelial cells compared to quiescent endothelial cells (Figure 4B) [34]. Furthermore, blocking of α6β1 integrin, a receptor for the basal membrane component laminin, hampered podosome rosette formation and significantly reduced MT1-MMP activity (Figure 4C). Blocking MT1-MMP activity using GM6001 and transfection of cells with siRNA completely abolished the ability for endothelial cells to sprout. In conclusion, blocking the α6β1 integrin may reduce sprouting due to a reduced MT1-MMP activity. This was demonstrated in an in vivo model involving highly angiogenic RipTag2 tumours [34]. Blocking of α6 integrin resulted in a significantly reduced density of endothelial rosettes, followed by a significant decrease in vessel branching. The suggested involvement of rosettes in tumour angiogenesis and the potential ability to block this process might be a target for development of therapeutic strategies to reduce tumour progression and possibly metastasis.

**Podosome-targeted therapy**

Since cell survival and angiogenesis are crucial factors in tumour progression, integrins that are important for the formation of podosomes might serve as an interesting target for cancer treatment.

For patients that do not respond to current treatment, it might be an option to target upregulated tumour-specific molecules instead. The integrin αvβ3 was found to be abundantly expressed on cancer cells and not on quiescent cells, which makes it an attractive therapeutic target [35]. Since the Arg-Gly-Asp (RGD) tripeptide sequence was proven to be specifically recognized by the αvβ3 integrin this could be utilized for therapeutic purposes [36]. Radiotherapy based on 177Lu-labelled dimeric RGD peptides (177Lu-3PRGD2) is an example of this, which was recently discovered and investigated by Jiyun Shi et al. [36]. Mice that received the targeted radiotherapy 177Lu-3PRGD2 showed significant tumour inhibition compared to saline-treated mice (Figure 6A-B). Mice that received 177Lu-3PRGD2 twice daily exhibited a better tumour inhibition compared to a single dose (Figure 6A). Also treatment with the anti-angiogenic drug "Endostar" showed a significant reduction of tumour growth compared to the saline-treated control group. Mice pre-treated with Endostar for five days before 177Lu-3PRGD2 administration, exhibited a similar degree of tumour inhibition compared to the group receiving both treatments at the same day (Figure 6C-D). Both therapy with 177Lu- 3PRGD2 twice daily as well as combination therapy showed inhibition of tumour growth. Since the combination therapy requires daily injections with Endostar, the two-dose 177Lu- 3PRGD2 therapy is more desirable.

**Invadopodia-mediated metastasis**

Metastasis can be described as a complex process during which primary tumour cells migrate to distant sites [38]. As reviewed by Fidler et al., this process consists of multiple steps: invasion through the surrounding ECM, intravasation into the bloodstream, transportation via the systemic circulation, and eventually extravasation and formation of new tumours at secondary sites [38]. Invadopodia-mediated ECM degradation is essential during the invasion, intravasation, and extravasation steps of metastasis [39]. ECM degradation by matrix proteases allows the primary tumour cells to migrate.

Invadopodia are able to degrade and remodel the ECM by the recruitment of specific proteases; secreted and membrane-bound MMPs, A Disintegrin And Metalloproteinase (ADAM proteases), and membrane-bound serine proteases. MT1-MMP is a member of the MMPs and plays, as described before, an important role in invadopodia-mediated ECM degradation and remodelling. Perentes et al. demonstrated that down-regulation of MT1-MMP results in a significant decrease in the occurrence of lung metastases which corresponds with reduced cancer cell migration and intravasation [40]. Invadopodia also recruit ADAM proteases, of which ADAM12 has emerged as a prognostic marker for breast cancer and plays an important role in matrix degradation [41]. The last group

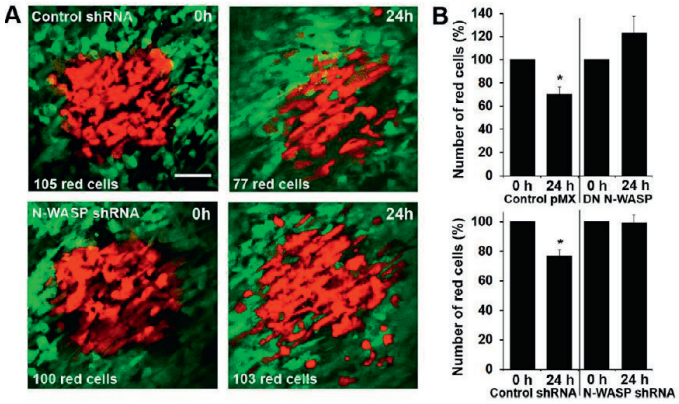


***Figure 5*** *In vivo intravasation assay of mammary adenocarcinoma MTLn3 cells requires the activity of N- WASP. (A) At 0 hours, the cells with the control shRNA vector at the top, and the cells with the N-WASP shRNA vector at the bottom were converted into a red state by the protein Dendra. At 24 hours, the same cells are shown, and there is more movement of the control shRNA MTLn3 tumor cells into the blood vessels. Scale: 70 µm. (B) Number of red cells remaining around the blood vessel of DN N-WASP and shRNA N-WASP tumours, normalized to the cell number at 0 hours. N-WASP: neural Wiskott–Aldrich syndrome protein. pMX: empty vector. DN: double negative, acts as competitive inhibitor of endogenous N-WASP, as it lacks amino acids for activation of the Arp2/3 complex (top graphs). shRNA: small hairpin RNA, to block N-WASP by silencing N-WASP expression (bottom part). Error bars indicate the SEM (standard error of the mean). * p<0.05 Figure adapted from Gligorijevic et al. [46].*

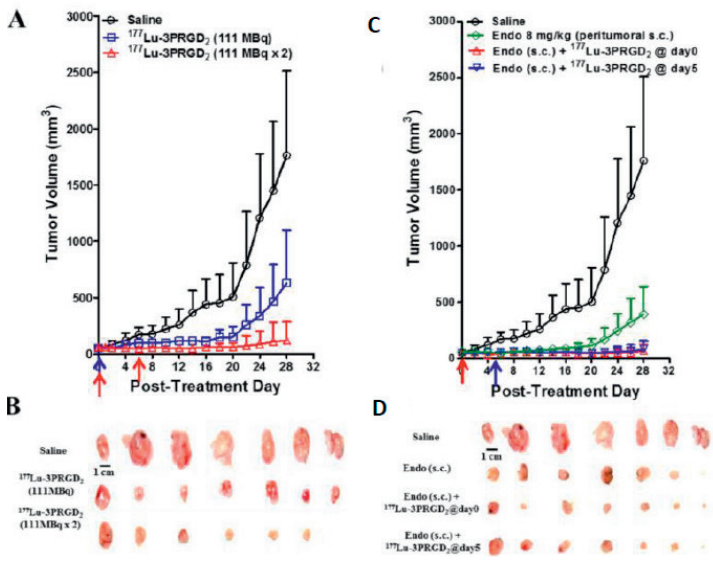


***Figure 6*** *Radionuclide therapy with 177Lu-3PRGD2 combined with Endostar. (A) Radionuclide therapy of established U87MG tumour in nude mice with saline (as control), 177Lu-3PRGD2 single dose (111 MBq), or 177Lu-3PRGD2 two doses (111 MBq × 2 on day 0 and day 6, respectively). (B) Tumour pictures of the groups depicted in (A) at the end of treatment. (C) Combination therapy of established U87MG tumours in nude mice with saline (as control), Endostar (8 mg/kg, peritumoral subcutaneous injection), Endostar (8 mg/kg, (s.c.) peritumoral subcutaneous injection) + 177Lu-3PRGD2 (111 MBq day 0), or Endostar (8 mg/kg, peritumoral subcutaneous injection) + 177Lu-3PRGD2 (111 MBq day 5). (D) Tumour pictures of the groups depicted in (C) at the end of treatment. The time point of administration of the radioactive compound 177Lu-3PRGD2 (111MBq) was indicated by an arrow, colours indicate corresponding graph. Volume of tumours in each treatment group was measured and expressed as a function of time (means ± SD, n = 7 per group). Figure was adapted from Jiyun Shi et al. [40].*

of proteases are the membrane-bound serine proteases. The serine protease Fibroblast Activating Protein-α (FAPα) was shown to be important in invadopodia-mediated matrix degradation and possibly cooperates with other proteases during this process. The exact role of most serine proteases is not elucidated yet [42]. Degradation of ECM is crucial for tumour cells to metastasize and therefore contributes to tumour progression [43].

To confirm the crucial role of invadopodia in tumour metastasis, it is important to visualize the direct degradation activity of invadopodia. Berginski et al. developed an in vitro model to visualize invadopodia by live cell imaging, although improvements have to be made to avoid false positive results and make this technique implementable [44].

Gligorijevic et al. investigated the importance of invadopodia in the invasion and intravasation steps of metastatic breast cancer by studying the role of Neural-WASp (N-WASp) in vivo [45]. Cancer cells with inhibited N-WASp function showed impaired invadopodia formation and a decreased invasiveness, which suggests that invasion of tumour cells is N-WASp dependent. This invasion appeared to be MMP dependent as well, since introduction of an inhibitor (GM6001) resulted in an impaired invasion of tumour cells in the ECM [45]. In addition to the invasion step, the activity of invadopodia during intravasation into the blood vessel was also investigated by Gligorijevic et al. [45]. Control tumour cells and N-WASp inhibited tumour cells were tracked with a fluorescent protein (Dendra2) to visualize intravasation. After 24 hours, there was no change (or even a minimal increase) in the amount of labeled N-WASp- inhibited tumour cells, which indicates that there was no migration of cells to the bloodstream. This suggests an essential role of N-WASP in the intravasation process (Figure 5). However, in this study, the effect of proliferation was not taken into account. Since proliferation of tumour cells might affect the cell count, the results may be unreliable.

Leong et al. investigated invadopodia and their contribution to in vivo extravasation by real-time 3D time-lapse imaging [46]. They showed that inhibition of cortactin (invadopodia initiation), Tks5 (maturation) and Tks4 (function) resulted in a decreased extravasation. This suggests that disruption of invadopodia via blocking of structural proteins leads to inhibition of metastasis, which provides direct evidence that invadopodia have functional roles during cancer metastasis.

**Invadopodia-targeted therapies**

Besides targeting components involved in angiogenesis, another target for anti-cancer treatment might be the metastatic process. Invadopodia enhance local invasion and metastasis and are therefore a potential target for the inhibition of cancer metastasis. Several proteins are involved in the regulation of invadopodia formation and function and are therefore interesting targets to inhibit invadopodia formation. DDGFRα activates Src tyrosine kinase to induce invadopodia assembly and thereby promote metastasis. For instance, the selective Src tyrosine kinase inhibitor SU6656 was found to decrease invadopodia formation, as well as the migration and invasion of human breast cancer cells [47]. PDGFRα expression has been identified as a tissue marker for survival in breast cancer patients [48]. The discovery of these important characteristics contributed to the development of several PDGFRα-targeted breast cancer therapies. Sunitinib (Sutent®, Pfizer) is a broad-spectrum tyrosine kinase inhibitor that inhibits PDGFRα amongst other targets. A clinical trial showed a positive effect of Sunitinib treatment in patients with late stage metastatic breast cancer [49]. However, since Sunitinib also targets several cellular components that play a role in invadopodia-independent metastasis, it is difficult to confirm whether the positive effects of Sunitinib treatment are indeed due to the inhibition of invadopodia dependent PDGFRα.

Some of the adverse effects induced by PDGFRα inhibitors might be prevented by the development and use of more specific PDGFRα inhibitors, such as humanized monoclonal antibodies. However, it is possible that highly specific PDGFRα inhibitors do not prevent the metastatic process sufficiently to be clinically beneficial. This implies that it is of crucial importance to obtain the right balance between specificity and efficacy when developing novel therapies targeting PDGFRα.

Another potential target might be MMPs since they have been associated with a poor clinical outcome in breast cancer patients [50-52]. Preclinical trials showed that targeting of several MMPs is effective in reducing invasiveness of cancer cells [53,54], whereas broad spectrum MMP inhibitors have not proven to be successful in clinical trials [55-57]. The low efficiency of MMP inhibitors in clinical trials can be due to the fact that several MMPs exert anti-tumour effects, which are impeded by using MMP inhibitors [58]. For instance, MMP-8 knock-out mice showed an increased incidence of skin tumours, which indicates a paradoxical role for MMP-8 in cancer [59]. Therefore, the strategy of broadly blocking MMPs to prevent metastasis may not be the correct approach, since this may also reduce the anti-tumour effects of certain MMPs. Specific MMP inhibitors might therefore accomplish better results. In addition, it might be interesting to target the system that delivers MMPs to the cellular location of invadopodia. Williams et al. showed that soluble N-ethylmaleimide-sensitive factor-activating protein receptor (SNARE) mediates the trafficking of MT1-MMP. Since trafficking of MT1-MMP is important for ECM degradation during tumour progression, SNARE mediated trafficking might be a potential target for the development of novel therapies [59].

Briefly, specifically targeting integrins or invadopodia-specific pathways might efficiently have a significant anti-tumour effect. Moreover, combining both (target) therapies can lead to even better results in cancer therapy.

## Discussion and future perspectives

In recent years, more and more research has focused on the role of podosomes and invadopodia in the processes of metastasis and angiogenesis, which have a considerable contribution to the high mortality seen in cancer patients.

Podosomes are mainly involved in physiological processes. However, they are also known to be involved in tumour angiogenesis. In contrast, invadopodia are specific for invasive cancer cells, but require similar signalling pathways as podosomes. As mentioned before, there is still no consensus reached in literature whether podosomes and invadopodia are similar or distinct structures. Since podosome rosettes are involved in tumour angiogenesis, they might be an interesting therapeutic target to reduce tumour progression and metastasis. It was demonstrated that blocking integrin α6β1 resulted in significantly reduced density of endothelial podosome rosettes, followed by a significant decrease in vessel branching. However, only a decrease in density was observed and not a full elimination of podosome rosettes. This suggests a possible role of escape routes in the formation and/or maturation of podosomes. The presence of compensatory escape routes might also explain the contradictory results that Reynolds et al. observed in β3 null mice, focusing on the involvement of β3 integrin in podosome formation. They showed enhanced angiogenesis, associated with an increased level of VEGF receptor 2 expression, suggesting that angiogenesis takes place in the absence of β3 integrin in these mice [60,61].

Based on the studies of Reynolds et al., it is suggested that αvβ3 has both pro- and anti-angiogenic properties. In the development of the most effective therapy targeting podosomes, complete understanding of the mechanisms and pathways of podosome formation is required. Therefore, contradictory results found in literature need to be elucidated. Overall, αvβ3 may yet turn out to be a good target for anti-angiogenic therapies that target the RGD sequence [37].

The use of novel in vivo detection techniques of the metastatic process may contribute to a better and a more reliable understanding of the different roles of invadopodia during metastasis, and may lead to the discovery of specific targets that might be interesting for the prevention of metastasis. Furthermore, several changes have to be made regarding the research strategy. To start with, there is a large gap between in vitro and in vivo experiments investigating invadopodia-targeted therapies. Only the early steps of metastasis can be investigated using current in vitro models. A tissue-engineered 3D in vitro model, which includes both ECM and blood vessels, might be useful to investigate the role of invadopodia in multiple steps of metastasis. In addition, the tumour environment and the methods of cancer induction in experimental models should resemble the human situation to improve external validity.

Another change that has to be made, is the development of more specific invadopodia-targeted therapies compared to the current non-specific inhibitors. Most therapies, such as PDGFRα- and MMP- targeted therapies, target multiple cellular pathways. This makes it difficult to attribute the potential therapeutic effect to the specific inhibition of invadopodia function. In order to confirm specificity of inhibitors, an invadopodia-specific biomarker should be developed. Furthermore, the implementation of invadopodia inhibitors from preclinical trials into clinical practice is another issue that need to be addressed. The timing of the treatment is crucial for its efficacy, since invadopodia inhibitors only prevent metastasis and do not influence the proliferation and growth of primary tumours. Invadopodia inhibitors can therefore be prescribed when there is (yet) no evidence of metastasis or to prevent metastasis of recurrent tumours.

Since invadopodia share characteristics and underlying formation pathways with podosomes, it is possible that invadopodia targeted therapy also affects podosomes. On top of that, podosomes are in turn involved in multiple physiological processes, therefore side effects may be expected.

Nowadays, a combination treatment of surgery, radiotherapy or chemotherapy combined with anti-angiogenic therapy is performed. Since not all cancer patients respond to conventional therapy, there is a need for other strategies to combat cancer. To improve current practice, treatment according to the 'personalized medicine' principle, for instance by using biomarkers, should be investigated. As it is known for breast cancer, in 20% of the cases there is overexpression of the HER2 gene which can influence the effectiveness of HER2 targeted treatments such as Trastuzumab [62-64].

## Conclusion

In conclusion, podosomes are important for angiogenesis, mediating cancer progression. Furthermore, invadopodia also contribute to this progression by promoting metastasis. Invadopodia are specific for invasive cancer cells and invadopodia-specific inhibitors seem to be promising in preventing metastasis of highly invasive cancers. Since podosomes show a lot of similarities regarding signalling pathways with invadapodia, these inhibitors might also affect podosome-mediated angiogenesis. Because of the important role of podosomes - and possibly invadopodia - in several physiological processes, the use of invado-

podia inhibitors might lead to side effects related to those processes. It is important to unravel the exact mechanisms of podosomes and invadopodia initiation and formation to clarify the involvement of these protrusion-like structures in the process of tumour progression. This can eventually contribute to the identification of novel prognostic markers and development targeted therapies. In addition to anti-cancer treatment with cytotoxic drugs, therapies preventing angiogenesis and metastasis might be beneficial in combating metastatic cancer.

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As RAMS aims to enlighten both students and professionals, we are proud to present a new element in our journal: the exam questions. The questions have been taken from exams from different bachelor modules. Find out if you can remember what you learned during the bachelor! The right answers and the number of students who answered correctly can be found further on in this journal.

***We challenge you!***

## EXAM QUESTIONS

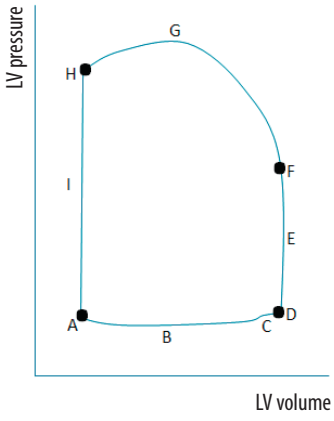
**Question 1:** The average volume of fluid that passes through the gastrointestinal tract of a young adult male on a daily basis amounts to about?

*Module Metabolism, water and mineral homeostasis*

a) 2 litres
b) 4 litres
c) 8 litres
d) 12 litres

**Question 2:** Acute rheumatic fever in childhood can lead to a severe mitral stenosis. As a result a heart murmur might be heard when blood passes through the mitral valve. During which section of the adjacent pressure-flow curve might this murmur be heard?

*Module Circulation and Respiration 1*




*The answers to these questions can be found on page 19 in this journal*

# THE SEARCH FOR AN EASIER AND FASTER WAY TO DETECT PANCREATIC CANCER

**Shelley Dalloyaux**[1]

[1] Bachelor Medical Student

"How could it be that despite all the new advances in science and exciting breakthroughs in technology, survival rates for pancreatic cancer have remained so astoundingly low? This is largely a matter of timing. Over 85 percent of all pancreatic cancers are diagnosed late, when someone has less than a 2 percent chance of survival. At this point, the tumors have usually spread and it is no longer possible to operate and cut them out. Why is pancreatic cancer being detected so late?" (Andraka, Breakthrough, p. 92) [1]

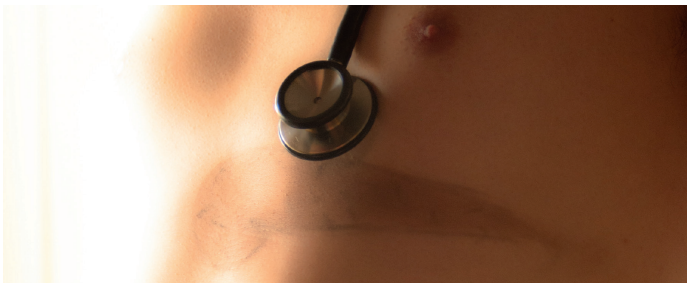
The young scientist, Jack Andraka, only fifteen years old, wondered precisely this when a close family member died of pancreatic cancer. It inspired him to develop a new way to detect pancreatic cancer in an early stage. Andraka had always been interested in science and had won several prizes with school projects, but he soon realized that starting up a research project without any professional resources is quite a challenge.

Andraka wanted to design a new, quick and cheap way to detect pancreatic cancer in an early stage. However, because most articles are not open-access, his search for relevant information was initially limited to Google and Wikipedia. During his search he found an article containing a database of 8,000 different proteins found in patients with pancreatic cancer. One of these could be a potential biomarker for detection in an early stage. However, to qualify as a good biomarker, a protein needs to fulfill certain criteria: (i) the protein should be up-regulated so its concentration is higher in patients with pancreatic cancer compared to controls; (ii) the protein needs to be expressed in an early stage of the disease and (iii) the protein has to be specific for pancreatic cancer only. Another requirement is that the protein must be present in blood or urine (iv) [1]. To identify a protein that met all the above-mentioned criteria he had to buy articles that were not open-access. This is expensive, costs a lot of time, and often these articles do not contain relevant information.

Mesothelin, a protein that is found in high concentrations in people with pancreatic cancer, seemed to meet the criteria to be used as a biomarker. The next step was to find a valid method for the detection of mesothelin. When reading an article about carbon nanotubes and following class about antibodies he came up with an idea for a detection method. Now he only needed the facilities and resources to test it, which is why he sent his research proposal to two hundred scientists. After a lot of rejections, Dr. Maitra from the John Hopkins University invited him over to talk about his plans, after which he decided to provide the lab space Andraka needed to do his practical work. Finally, after seven months of successes and failures, he managed to develop a cheap, fast and safe test to detect pancreatic cancer.

So far, the first test results look very promising. His methods appear to be faster, less expensive and more sensitive than the current gold standards. However, it might take years before this new test will become available for use as it has not yet been tested on patient samples and still needs approval from the Food and Drug Administration [1].

However, Jack Andraka is not the only one searching for a method to detect pancreatic cancer. Another research group, led by dr. Tatjana Crnogorac-Jurcevic, found a three-protein biomarker panel that can detect early-stage pancreatic cancer in patient's urine. They published their findings in a journal of the American Association for Cancer Research [2].



The presence of a combination of the three proteins, LYVE-1, REG1A, and TFF1, is a marker for stage I-II pancreatic ductal adenocarcinoma in patients with over 90% accuracy. The diagnostic performance needs to be tested further, but the fact that there are two new detection methods for pancreatic cancer in development can be seen as a big step forward in the medical world, potentially having the ability to save lives.

Most likely, no one would expect that a fifteen year old boy would be able to detect a new method for the detection of pancreatic cancer. However, although his method is not yet in use, and despite that other researchers were able to develop another reliable test, the story of Jack Andraka shows that even when you start out with little knowledge and experience, you can create impact with research that furthers science and generates new research questions. As long as you keep trying, even when things do not go the way you want them to go, you could do things you never imagined you could.

Furthermore, his story illustrates the difficulties and inaccessibility that characterize our scientific world of today. As long as the majority of scientific articles are not open-access, this will not change. In the Netherlands, several universities, including the Radboud University, are committed to increase the amount of open-access articles. For example, last October, an "International open access week" was organized to raise awareness for this problem. However, before articles can be freely accessed, and thus open up the scientific world, a higher level of quality control of articles and new methods of funding are necessary. When this is achieved, the amount of articles published open-access can be increased, to everyone's benefit.

*Andraka's research won the George E. Moore Award in May 2012 during the Intel International Science and Engineering Fair in Pittsburgh, Pennsylvania.*

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# THE PIRIFORMIS MUSCLE SYNDROME: A CLINICAL LESSON

**Carla Timmer[1,3], Prashant Komdeur[2,4]**

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**ABSTRACT:**

In the clinical lesson, we present a patient with piriformis muscle syndrome (PMS), an entrapment neuropathy of the sciatic nerve by the piriformis muscle. PMS is a controversial syndrome, currently without a comprehensive treatment protocol. Symptoms include radiating back and buttock pain during physical activity, such as running. Different diagnostic tests which can be used during physical examination are described. Additional diagnostic test and treatment options are reported. Treatment should include physical therapy focusing on strengthening core stability. Our patient recovered after 4 months of physical therapy.

**KEYWORDS:** Sciatica, piriformis muscle, Piriformis Muscle Syndrome

## Introduction

The Piriformis Muscle Syndrome (PMS) is a controversial condition that is still a subject of much discussion. Currently there is no consensus about the existence of this syndrome. This contributes to the fact that there is no uniform definition or method to diagnose PMS. Treatment is also without a comprehensive protocol.

In recent publications, PMS is described as an entrapment neuropathy of the sciatic nerve entailing radiating pain in the gluteal region as a result of compression of the nerve by the piriformis muscle. In this clinical lesson we will describe a case of PMS and report the current possibilities in diagnosing and treating PMS.

## Case

A 32-year old runner presented to our clinic with persistent complaints of lower back pain and pain in the left buttocks. These symptoms had been present for 2 years. Sometimes the pain radiated to the left leg. Symptoms were aggravated by running; normally, he could run 12.5 kilometers without complaints. Now his pain started during physical activity such as running and would continue for some hours following the activity. When visiting our clinic, he had stopped running to see if this would relieve the injury, which was not the case. There was no pain at rest.Patient had previously been referred to the neurologist; there was no significant compression of nerve roots on MRI of the lower back.

At physical examination, we found a normal range of motion of the lower back, hips and knees. Palpation of the left sacro-iliacal (SI-) joint was slightly painful but mobility was normal. Pain could be elicited by putting pressure on the left piriformis muscle by direct palpation and stretch on this muscle provoked the familiar pain known to the patient. Functional testing using the step-down test showed a hip drop and knee motion to the medial side, a motion called 'kneeing in' showing lack of strength of the hip abductors. Further core stability testing showed a lack of neuromuscular control around hip and core.

## Background

### Anatomy

The piriformis muscle, also known as the pear-shaped muscle, originates from the sacral bone at S2-S4 and inserts on the greater trochanter of the femur (Figure 1). When the hip and knee are extended the muscle functions as an exorotator of the hip joint. However, its main function is stabilization of the hip [1].

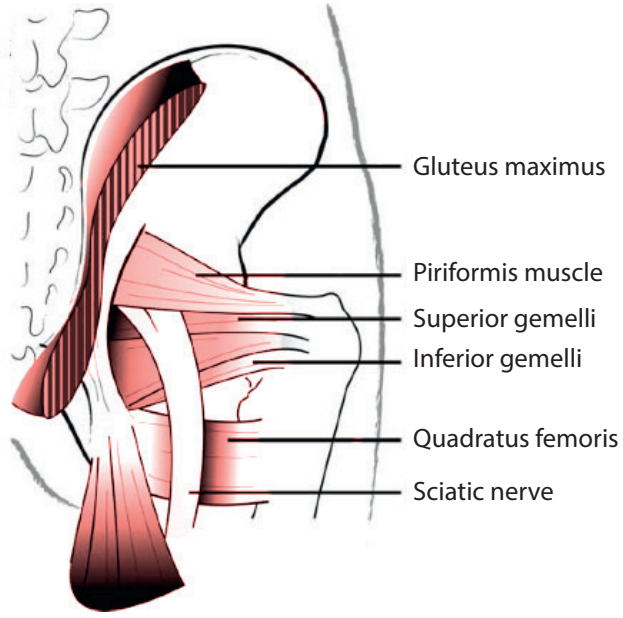


***Figure 1*** *Anatomy of the piriformis muscle and the sciatic nerve showing their close relationship*

Immediately caudal of this muscle, the sciatic nerve runs through the greater sciatic foramen of the pelvis. It originates from the spinal nerves L4-S3 and after splitting into the common peroneus nerve and the tibial nerve at knee-level it ends in the foot. The sciatic nerve supplies the sensory innervation of the skin of the majority of the lower limb and also innervates the lateral rotators and hamstring muscles.

### Epidemiology

Due to lack of a definition, the reporting of reliable prevalence or incidence percentages proved to be difficult. Of all patients with sciatica (back or gluteal pain with radiating pain), only a small percentage suffers from PMS; reported estimates of this percentage range from 1 to 15% [2-4]. Risk factors for developing PMS seem to include female gender, practicing endurance sports with mainly forward movements

of the lower extremity (i.e. cycling or running), and sports with frequent jumping combined with exorotation of the extended leg (i.e. ballet).

**Pathophysiology**

There are multiple hypotheses concerning the etiology of PMS. One of these, as mentioned in the introduction, states that PMS is caused by direct excitation of the sciatic nerve resulting from compression by the piriformis muscles in the infrapiriformis canal [2-4]. The most frequent cause of PMS is traumatic injury; scarring of the infrapiriformis canal after blunt trauma. Non-traumatic causes include surmenage following intensive exercise, abnormal mobility of the SI-joint and hyperpronation of the foot. Some case reports also mention external compression, by a wallet for example, as a cause of PMS [3,5]. Anatomical variations in the relationship between the sciatic nerve and the muscle fibers of the piriformis muscle do not predispose for development of PMS [1-4, 6,7].

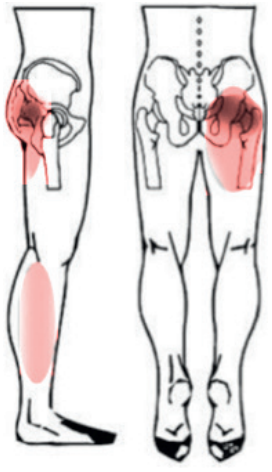

***Figure 2*** *Typical location of the radiating pain associated with piriformis muscle syndrome on the lower back, buttock and upper leg.*

Other structures in the pelvis might also be responsible for complaints similar to PMS. Radiculopathy and discopathy should always be excluded. Compression of the sciatic nerve may also be caused by the internal obturator muscle or the gemelli muscles. Nerve compression by tumors of different origins (vascular, intestinal or gynaecological) can also cause pain as seen in PMS. Arthritis of the hip joint or SI-joint might be a primary or secondary (through surmenage of the piriformis muscle) cause of PMS-like pain [1-4, 6, 7].

## Clinical presentation

Patients complain of a deep pain in the gluteal region, uni- or bilaterally. This pain often radiates to the dorsal and, in some cases, also the lateral side of the upper leg (Figure 2). Complaints are provoked by prolonged sitting and intensive strain of the piriformis muscle, for example during ballet dancing or other activities that require extensive hip stabilization. Typically, pain free intervals occur during the day [2, 3].

The following signs during examination of the locomotor apparatus can point the examiner in the direction of PMS. The patient might position the affected leg with a slight flexion in the knee and exorotation when supine; in this position the piriformis muscle is relaxed. Palpation of the piriformis muscle, which can be done by following its course through the buttock area while rotating the hip, on the ipsilateral side is more painful when compared to the contralateral leg. Neurologic abnormalities do not rule out PMS [1-4, 6, 7].

A number of clinical tests or maneuvers provoking strain on the piriformis muscle, have been described to help the examiner make the diagnosis PMS more probable. The passive tests, Frieberg and FADDIR test, are performed by the examiner. The Frieberg test (Fig 3b) involves internal rotation of the hip with the patient in a prone position. The FADDIR test (Fig 3a) is performed by simultaneously flexing, adducting and internally rotating the hip of the patient. The Pace and Beatty test are actively performed by the patient. The Pace test (Fig 3c) includes active adducting of the hip while the patient is in a sitting position. During Beatty's test (Fig 3d) the patient is in prone position and is asked to adduct the hip while the knee is flexed 90 degrees. The maneuvers are considered positive when the recognizable pain is elicited. While performing these tests, it is important to hold the positions for at least 10-20 seconds [1-4, 6, 7]. The diagnostic value of each test individually is not clear, but each positive test should help guide the examiner towards the possible diagnosis of PMS.

## Diagnosis

**Imaging studies**

Imaging is essential in excluding other causes of irritation or compression of the sciatic nerve. Degenerative changes of the hip or SI-joint can be ruled out by conventional X-ray. An MRI of the lumbar spine is necessary to exclude nerve root compression; although MRI also shows possible hypertrophy or atrophy of the piriformis muscle, this does not prove PMS [1, 2, 6]. MR-neurography, a MRI-scan with special settings, can show irritation of the sciatic nerve [1, 2].

**Electromyography**

Electromyography (EMG) of the affected leg might show a delayed conduction of neurological activation. Change of position of the leg, from a neutral position to the position as used during the FADIR test (Figure 3a), can also affect nerve conduction [1-4, 6]. However, it remains unclear how to interpret these delays, and thereby the diagnostic value of EMG for PMS is not yet established [8]. EMG can also be used to exclude radiculopathy and other forms of entrapment of the sciatic nerve [1-4, 6, 7].

## Treatment

**Physical therapy**

In general, physical therapy (PT) is the first step in treatment of PMS. PT can be combined with pain medication, when needed. PT involves daily stretching and relaxation of the piriformis muscle. Deep tissue massage or friction of the muscle can be used to relieve tension and remove adhesions. Training of other exorotators of the hip is essential. When inadequate core stability is present, PT should also focus on strengthening muscles necessary for core stability. Movements and activities that have been known to provoke symptoms should be avoided [1-4].

The controversy surrounding PMS, the lack of a clear definition and a golden standard for diagnosis, makes the comparison of different treatment regiments used in scientific research papers extremely difficult. For PT, the variation in regimes and variation in intensity of the provided guidance, makes evidence based research of a clear treatment regime almost impossible. It is, however, evident that PT, as described in the previous paragraph, combined with medication (paracetamol and/or non-steroidal anti-inflammatory drugs), significantly reduced symptoms for a large number of patients [1-4, 6, 7].

**Intramuscular injections**

Injections with botulinum neuroxine (Botox) can be used to relax the muscle fibers of the piriformis muscle. Guidance, through sonography, MRI or CT, ensures the correct position of the injection; without

guidance the majority of intramuscular injection are not administered at the desired location. Almost half of the patients report a reduction of complaints after injection with Botox [2]. When indicated, by a clear and prolonged result, injections can be repeated [2-4]. In a similar way, a guided injection with analgesics or corticosteroids can be administered. The exact location of the injection to achieve maximum results, be it the punctum maximum of the pain, or in the course of the piriformis muscle is not yet known [1-4].

**Surgery**

Surgical intervention is often the last step in treatment of PMS, after conservative methods had unsatisfactory results. A common technique is a tenotomy of the insertion of the piriformis tendon on the greater trochanter. During the procedure any adhesions of the fascia on the sciatic nerve can be removed. In many cases, a satisfactory result is achieved following surgical treatment of patients with PMS [1-7].

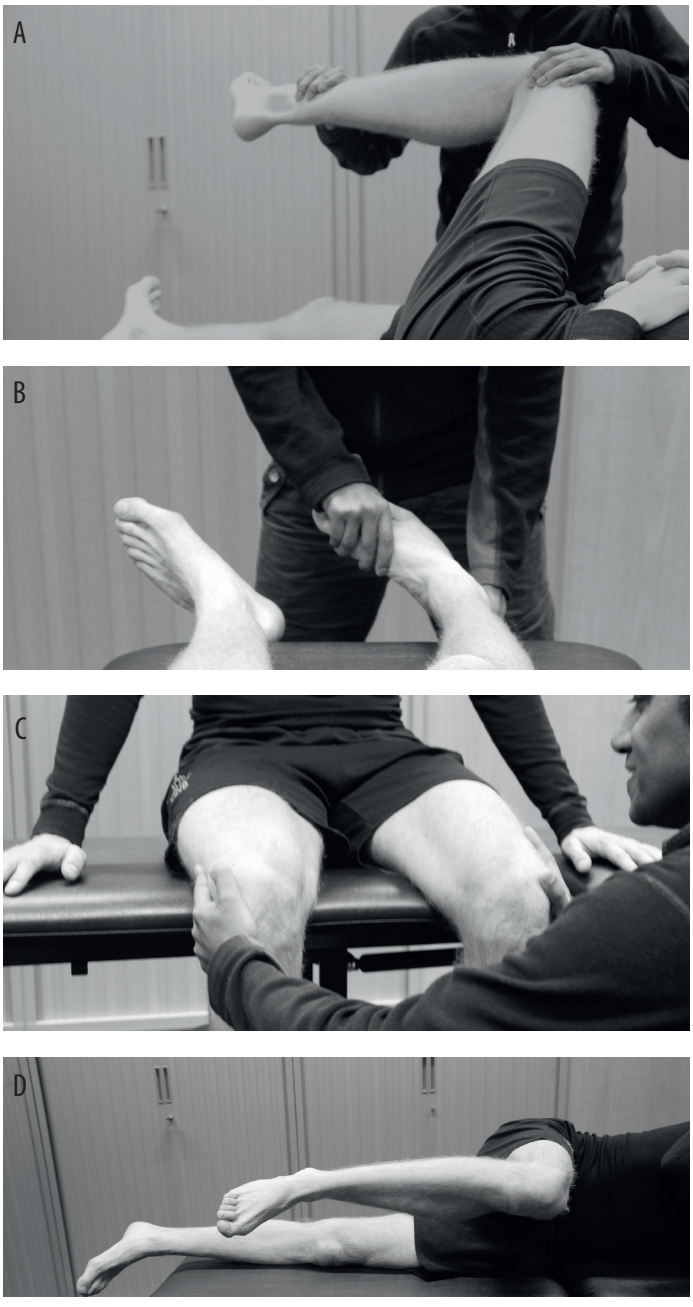


***Figure 3*** *Clinical tests and maneuvers eliciting strain on the piriformis muscle:*
*A - FADDIR test (flexion, adduction and internal rotation of the hip)*
*B - Freiberg test (internal rotation of the hip in prone position)*
*C - Pace test (active adduction of the hip in seated position)*
*D - Beatty's test (adduction of the hip with knee in 90 degrees flexion in prone position)*

## Case - continued

The aforementioned patient had a clear lack of core and functional stability. Together with the pattern of radiating pain we considered PMS as diagnosis. We did not perform any additional testing, since other nerve entrapment or compression was already ruled out by MRI. Due to the controversy regarding the treatment of choice, we advised the patient to start with PT. He started with core and functional stability training at our sports medical centre, in combination with stretching and deep friction of the piriformis muscle. After 4 months of treatment, he could start running again and his back pain and buttock pain were almost gone.

## Conclusion

When confronted with a patient complaining of radiating back or buttocks pain, PMS should be considered as a possible diagnosis. Other possible causes, including nerve root compression, should be excluded with a MRI of the lower back and conventional X-ray of the pelvis. Painful palpation of the piriformis muscle and positive FADDIR, Freiberg, Pace and Beatty tests could further assist physicians and physical therapists in their diagnostic process. While treatment options vary, it seems clear that physical therapy should be the first step in treating PMS. Further research on the prevalence, diagnosis and treatment of PMS could also help therapist to treat patients with lower back pain.

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# NEUROTOP: GETTING TO KNOW THE NEUROLOGISTS AT RADBOUDUMC AND GETTING THEM TO KNOW YOU

**Jasper M. Maters**[1]

[1] Master Medical Student

Every summer, the Department of Neurology at Radboudumc hosts a five-day summer school in 'Huize Heyendael', called Neurotop. All medical students who have successfully completed the third-year course 'Zenuwstelsel' (neurology) can apply, from which ten students are eventually selected. Participants follow an intensive week-long program, full of lectures and clinical demonstrations. A master class aimed at increasing confidence during presentations is given by Anne Spies from Spies & Spreken. Additionally, they are asked to write a research proposal with the help of one of the neurologists. The participant with the best results wins the opportunity to carry out his or her proposal.

One neurologist who is involved in Neurotop is Baziel van Engelen, a professor of neuromuscular diseases. Between 1975 and 1984 Baziel received his MD in medical science and studied philosophy at the University of Amsterdam, from which he graduated cum laude in 1992. Since then, he has been involved in numerous studies about myotonic dystrophies, facioscapulohumeral muscular dystrophy, and inflammatory myopathies, including clinical trials in patients with neuromuscular disorders. When Neurotop was realized in 2007, he was one of the initiators. To elucidate the ideas behind Neurotop and his own work as a researcher, we conducted the following interview with him.

**When and why did you set up this summer school?**

We initiated Neurotop with the aim of passing something on to the younger generation, thereby searching for the future of our profession. We believe the future is determined by young doctors and we would love to meet them. Therefore, Neurotop is the ideal way to achieve this in both a formal and informal way.

> ***"We initiated Neurotop with the aim of passing something on to the younger generation, thereby searching for the future of our profession."***

I really enjoy working with young professionals. The best part is to see them growing in the process. You witness them losing themselves in a subject and eventually getting the hang of it. They make commitments, come up with new ideas and reach out to other professionals, all in order to make progress in their research. I am constantly surprised by all the initiatives that are taken, this also applies to the PhD students I advise. I compare a PhD trajectory with a walk through the Veluwe. During the first months a PhD student can follow his own route as long as results are obtained. At a certain point we look how far this person has wandered off and what their trip has brought so far, and then we walk to the exit together.

When someone is really creative and is able to work independently, that person is granted a lot of freedom but if someone needs more guidance, I am there to help.

**How does the selection take place?**

The candidates are selected based on resume, content of the letters we receive and their grades. We wish to select individuals who are motivated, talented and have participated in extracurricular activities. We are aware that it is a lot to ask from young students, so for a great part it is about potential and open mindedness. This requires personal growth in areas other than the academic one.

**The master class given by Anne Spies appears to be the main focus of Neurotop. Why is that?**

No, that is not the case. The focus lies on the individual: we would like to get to know you as a person. Students need to realize that neurology is a 'way of life' which needs to suit you as a person.

> ***"We want Neurotop to be broader than the left hemisphere."***

The bachelor is mainly focused on intellectual capacity, whereas we want to explore students' capacities in other areas. Anne Spies (presentation coach) ultimately attempts to make you discover who you are while standing in front of an audience. The question they need to answer is: *What is your style?* Therefore, we see the master class training as a form of 'bildung' (education). We want Neurotop to be broader than the left hemisphere.

**Neurology is a medical specialization that needs to suit you, what type of a person is suited to being a neurologist?**

There are all kinds of neurologists, it has become a specialism with many faces. While it used to be a thoughtful and considerate man, it is now more diverse. There are neurologists who perform acute interventions such as thrombolysis, but also other colleagues who focus on age related afflictions like dementia and work together with geriatrists. Additionally, other neurologists occupy themselves with specific diseases, for example, Parkinson. Of course, neurology remains a profession which requires a studious mind, which is also the fun part. You have a certain amount of knowledge or a certain skill at your disposal and try to help someone by applying it, driven by empathy. But there is no longer just one type of neurologist .

**What is the most appealing aspect of neurology?**

It is a great profession because it is really clinical and diagnosis is mostly made based only on conversing with the patient and the accompanying neurological examination. Modern techniques such as MRI and histological research are gaining importance but it remains mainly a clinical specialism. Being a good neurologist requires you to have an analytic mind, as it is about solving puzzles. Neurology balances on the border between somatic medicine and psychiatry. All considering, your nervous systems decides what you do, how you look (muscle architecture) and who you are. It is about identity.

***Figure 1*** *Left: Baziel van Engelen, professor of neuromuscular diseases and one of the initiators of Neurotop in 2007. Right: participants of the Neurotop Summer School, organized by the Department of Neurology at Radboudumc.*

**Neurology must be an interesting field of research. Could you tell us about your own passion for research?**

The best part about research in general is the process of creative thinking by which you try to find original solutions for a problem. Various tools are applied including biological, histological and physiological ones. In addition to that, the great fact about being a physician is that you conduct research in order to actually treat patients. Therefore, we are really capable to apply the gained knowledge into practice. Although, it is important to mention that you work together with individuals who may have other perspectives than yours: molecular biologists, genetic engineers and rehabilitation physicians. The key is to reflect on these perspectives and try to solve the puzzle. Occasionally, this involves a total surprise: the solution was there all along and just by viewing it from another perspective you are suddenly able to see it. All in all, research is wonderful when it brings you to an optimal treatment, especially when driven by your own curiosity.

**Let us get back to Neurotop. The participants need to write a research proposal. How do you rate these considering the proposal needs to be finished in quite a short amount of time?**

Everyone is advised during the writing process In general, there are different topics from which the participating students can pick. In the end, we judge both the presentations and structure of the proposal. We ask ourselves questions like: has this person succeeded in mastering the subject in a few days? Was this individual really invested in their proposal? We love seeing students getting more and more excited about their topic, and to see their own ideas and insights.

**What have the winning research proposals brought over the years?**

In fact we have several winners, as it often happens that a student and his mentor keep working together regardless of whether their research proposal has won or not. Also, some students who started with a subject in Neurotop have decided to continue on and do their PhD programme on this subject, because of which they have eventually acquired a residency. Every participant is coached by staff members. If they work well together, they can choose to continue working, as has been the case for several students in the past. For us it is wonderful to be able to bond with our future colleagues and for students it is interesting to be noticed as an individual instead of being one of three hundred anonymous students. This can be realized further by attendance of extracurricular lectures and clinical demonstrations by students.

**What are the (other) options available for students that are interested in neurology in their bachelor and master?**

As a student several options are available, for example: They are able to pick an elective course involving neurology or can choose to do their scientific internship in neurology. However, everyone can have their own approach depending on what drives them. I would advise them to try to contact a teacher who inspires them. What strikes me is the fact that many students are interested in research, but not very interested in education. This is a shame because with an interest in education it is possible to try to aid us in improving the education program. Although contributing to patient care is harder for students in their bachelor, it is possible to complete your bachelor internship (at the outpatient clinic, during their second year) at our department. I love these kinds of internships because it allows me to reflect on my own behavior. Young students might not know that much about neurology, but they can observe how I communicate with patients and tell me whether they would do it the same way.

Are you interested in research and would you learn more about neurology? Every third-year student will receive an email about Neurotop around February and the deadline for application will be around May. So take your chance and sign up!

More information can be found on:
https://www.radboudumc.nl/Research/Organisationofresearch/Departments/neurology/Pages/Neurotop.aspx

For additional information on Baziel van Engelen, be sure to check out his webpage, available at the following address:
http://radboudumc-neurologie.nl/medewerker/prof-dr-baziel-van-engelen/

# CHANGES IN THERAPY PERSPECTIVE OF THE MOST COMMON AND AGGRESSIVE PRIMARY BRAIN TUMOUR

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**ABSTRACT:**

Glioblastoma (multiforme) is the most common and aggressive primary brain tumour. Median survival is very poor and much research is being done on innovative therapies to improve both quality of life and survival. In this review, recent articles on glioblastoma therapy are addressed and discussed. Standard therapy consists of a total tumour resection combined with radiotherapy and chemotherapy, but many new fields including gene therapy and nanomedicine may alter standard therapy in the future. However, much more investigation is needed before these innovative therapies can be implemented in patient care.

**KEYWORDS:** Glioblastoma multiforme, glioma, therapy, innovation, epigenetics, gene therapy, metabolic therapy, nanomedicine

## Introduction

Glioblastoma multiforme or simply glioblastoma is defined as a grade IV astrocytoma, making it the most malignant form of astrocytoma. Astrocytomas are tumours derived from astrocytes, which are supporting glial cells in the central nervous system (CNS). Men are more commonly diagnosed with glioblastomas than women and present with the disease mainly between the ages of 45 to 65 [1]. Glioblastomas are the most common primary brain tumours and have an appalling median survival of less than a year. This emphasises the importance of research that explores innovative therapies [2] [3].

### Symptoms and diagnosis

Glioblastoma can manifest itself in various ways. Glioblastoma can occur anywhere in the brain and symptoms depend strongly on tumour location and invasion. Therefore, specific neurological symptoms between patients differ greatly, but can sometimes point the physician towards the affected brain area. Examples of these specific symptoms are loss of vision, ataxia and aphasia. Other symptoms are less specific and can be explained by an increase in intracranial pressure as a consequence of rapid growth of the tumour. Nausea, vomiting, seizures and cognitive dysfunction are frequently reported [4]. Even after diagnosis, and unfortunately also during treatment, symptoms can change as a consequence of glioblastoma expansion, underlining the significance of frequent follow-up. Examples of these are more intense headaches, lethargy and even tonic-clonic seizures, which require critical care [5].

Diagnosis of glioblastoma consists of the aforementioned neurological examination as an initial indication, followed by a CT or MRI scan. Differentiation of primary brain tumours is virtually impossible with imaging alone and can only be confirmed using histological results. Magnetic resonance spectroscopy can provide an indication of the aggressiveness of the tumour by assessing metabolic activity [1].

### Therapy

Although glioblastomas are, as a rule, malignant, they do not usually invade organs outside of the CNS. However, they do invade brain tissue [1]. If possible, therapy is primarily focused on removing the tumour source by total resection, but if not, therapy is used to reduce the speed of tumour spreading. Obviously, this is not curative and it is therefore essential to develop new therapy techniques. In this clinical review article, outcomes of recent reviews will be combined to assess the different possible therapies to beat glioblastoma.

Glioblastoma is a very invasive form of glioma and requires immediate action after diagnosis. Current standard therapy is a combination of total surgical resection, radiotherapy and chemotherapy (mostly the cytostaticum temozolomide) [6]. In the elderly, combined therapy of temozolomide and hypofractionated radiotherapy is the preferred and evidence-based method, as opposed to surgery [7]. With respect to radiotherapy in glioblastoma, physicians should be aware of pseudoprogression. An early evaluation of tumour response to radiotherapy could show an increase in tumour size that is actually oedema caused by radiation effects or therapy-induced ischaemia [8].

Regarding surgery, it has been shown that survival is improved after a radical resection. Most contemporary neurosurgeons improve their resection success rate by utilizing new techniques, varying from easy-to-use to highly complicated. One example of this is the 5-ALA technique, in which a compound of porphyrin synthesis, 5-aminolevulinic acid, is used to elicit an accumulation of fluorescent porphyrins in glioma cells. Under blue light, a used and proven method for the past ten years, the neurosurgeon can distinguish normal brain tissue from the malignant tissue, resulting in a more accurate resection and survival [9] [10].

## Methods

PubMed was searched for English reviews on glioblastoma concerning humans. Because this review article addresses new, innovative therapies for glioblastoma, only articles published in 2014 or later were included. The search term "("glioblastoma"[MeSH Terms] AND (Review[ptyp] AND hasabstract[text] AND ("2014/01/01"[PDAT] : "3000/12/31"[PDAT]) AND "humans"[MeSH Terms] AND English[lang])" resulted in 37 articles, of whom 8 were included after availability selection and rigorous abstract scanning on potential and innovative character of the investigated therapy.

## Changes in perspective

To improve survival, the field of glioblastoma is subject to various developments in drug therapy. First of all, there are drugs currently under investigation as an addition to the standard therapy. Talampanel, for example, is a non-competitive antagonist of the AMPA-receptor, expressed on glioma cells. In phase II trials, talampanel inhibited tumour growth by mostly unknown mechanisms; more voluminous phase III trials are planned [2]. Upregulation of MET (a receptor tyrosine kinase) is seen in glioblastoma, inducing DNA synthesis and cell proliferation. Blocking MET and/or its ligand, HGF (hepatocyte growth factor), results in less

tumour growth. Therefore, the HGF antagonist NK4 and chimeric U1snRNA/ribozyme, an inhibitor of HGF and MET expression, are examples of potential therapy targets in glioblastoma and show promising results in phase II studies [11].

Secondly, epigenetics are of increasing interest. Recent research has shown that histone deacetylase inhibitors (HDACis) inhibit oncogenes and upregulate the expression of tumour suppressor genes. In addition, they inhibit angiogenesis [12]. However, antiangiogenic therapy (especially bevacizumab) has only been shown to improve progression-free survival, without affecting survival itself [13].

Moreover, significant advances in gene therapy are being made, but the standard therapy most likely will not change in the short term, because gene therapy faces many challenges. Firstly, delivery to the tumour is often compromised by the blood-brain barrier and the high intratumorous pressure. In addition, the lack of cell selectivity remains a problem. Since recently developed oncolytic viruses and nanoparticles are not tumour specific, damage to healthy brain tissue is an important adverse effect [14].

The experimental use of metabolic therapy is uncertain and even controversial, but glioblastoma cells might be influenced by reducing glucose and glutamine intake, two important nutrients for cancer stem cells. The calorie restricted ketogenic diet (KD-R) is believed to inhibit angiogenesis, inflammation and cell survival [15].

Advancements in nanomedicine are highly anticipated, but the field has only just started to get involved and faces quite a lot problems. For instance, when magnetic nanoparticles are injected into the tumour and the patient is placed in an alternating magnetic field, the particles accumulate in the tumour and thereby ablate the malignant tissue thermally. However, overheating of the particles can occur and lead to damage of otherwise healthy surrounding tissue [3] [16].

## Conclusion

All in all, the future in glioblastoma therapy seems promising. Standard therapy for glioblastoma is still the first choice and can be supplemented with novel therapies currently under investigation. These include antagonists of (over)expressed receptors (e.g. MET, HGF and AMPA) in cancer cells and antiangiogenic therapy. Although the latter does not affect overall survival, it does improve progression-free survival. Advancements in innovative fields, such as nanomedicine and gene therapy, are being made, but will most likely not alter glioblastoma therapy in the near future.

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*Curious to see how the 5-ALA technique is executed precisely? Many videos are to be found on YouTube, including a video recorded at the Neurosurgical Center (NCCN), Radboudumc [10].*

**CORRECT ANSWERS TO THE EXAM QUESTIONS**

**Question 1: 8 litres (Answer C, 60% answered correctly)**
People have a daily fluid intake of about 2 litres. Also, on a daily basis the exocrine glands secrete about 7 litres of enzymes, electrolytes and water into the digestive tract. (Human physiology : an integrated approach, 6th Edition / Dee Unglaub Silverthorn)

**Question 2: During diastole (Section B, 89% answered correctly)**
Rheumatic fever is an infection of group A streptococci that generally affects the heart, central nervous system, skin and joints. It is preceded by an pharyngeal infection and most often seen in 5 to 15 years olds. Due to the use of antibiotics and better hygiene, the number of patients with streptococcal infections and rheumatic fever has dropped. (Kumar & Clark 's Clinical Medicine, 7th Edition / Kumar & Clark)

# LITERATURE SEARCH:
# PRACTICAL GUIDELINES FOR MEDICAL STUDENTS

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[1] Master Medical Student

A key aspect of science is knowledge, of which the sharing is essential. The past thirty years have seen increasingly rapid advances within the field of information and communication technology. Medical knowledge is no longer limited to books and journals, but also extends to the internet. These days user-friendly search engines and databases are available to make it easier to obtain knowledge. In this article we will take a closer look at these sources with the objective of presenting some practical tips on how to obtain useful medical literature in an efficient way.

## Orientation

During our studies we regularly encounter topics in which we are interested and would like to explore in more depth. For example, you are a medical intern and you are going to see a patient with polycystic liver disease. It is possible that you have never heard of this disease as it is relatively rare. Therefore, you would like to know the incidence, symptoms, prognosis and treatment of the disease. Based on your personal preference, there are a few simple ways to obtain this information.

First off, Google, a frequently used search engine that is quick and easy to use. However, the trust worthiness of information found in this manner is questionable, especially in comparison to peer reviewed media. This may lead to the use of Wikipedia. Wikipedia: www.wikipedia.org is an open-access online encyclopedia that invites contributions from its users. It is operated as a charity by the non-profit Wikimedia Foundation. Therefore, the information found on Wikipedia can be written by anybody, meaning the accuracy of medical information on Wikipedia and its suitability as a learning source arguable [1, 2]. However, many physicians (and medical students) use it because it is easy and fast [3].

Let us take another look at polycystic liver disease. The English Wikipedia page only describes genes that are linked to the disease and polycystic kidney disease as an associated disease [4]. The Dutch Wikipedia page, surprisingly, does elaborate and describes incidence, symptoms, mechanism and treatment of the disease [5]. However, as it is in Dutch, it is not of much use to the rest of the world. Moreover, sometimes Wikipedia pages are not updated for an extensive periods of time. In conclusion, although Wikipedia can give you a good first impression, something that guarantees more reliable and recent information in needed.

Another option for some basic information can sometimes be found on patient information sites such as thuisarts.nl (in Dutch). More detailed information on pathophysiology, symptoms and possible treatments can be found on UpToDate: www.uptodate.com, an evidence based medical website. This website provides reviews on many medical conditions. The reviews are published by physician authors, editors and peer reviewers. Additionally, a patient version of the website that provides more detailed information than Wikipedia is available. For instance: the incidence and prevalence of a disease, but also more specific information concerning side effects of therapy. One disadvantage is that information about treatment options is written according to United States guidelines and is therefore not always applicable to Dutch patients.

The methods described above are all websites. However, there is nothing wrong with picking up a book from time to time, beside the fact that information in books is less frequently updated than information on most websites. Every university and most hospitals have their own library.

Sometimes in evidence based medicine you require answers to questions that cannot be found in any of those resources. Next, Let us get a closer look on how to obtain peer reviewed medical literature.

## PICO

Formulating a question is the first and most important step in any type of research. However, it can also be the most difficult step. In medical research, the PICO-method can be used to formulate a question. PICO is an acronym for patient, intervention, control and outcome. By describing and combining these four components you can put together a complete research question for your search. We will explain the PICO method using an example from the clinic.

Imagine you are a medical intern at the department of obstetrics and gynecology, you are going to see a pregnant woman in her third trimester of pregnancy. On ultrasound the fetus appears to be macrosomic. Vaginal delivery of a macrosomic fetus increases the risk of shoulder dystocia; a serious complication by which one or both of the fetal shoulders are stuck above the pelvic outlet. Shoulder dystocia can lead to brachial plexus palsies, clavicular fractures, humeral fractures, fetal asphyxia and even fetal death. The risk of shoulder dystocia increases with the weight of the fetus. Therefore you wonder if you should induce vaginal birth at an earlier point in time, so the child will weigh less at the time of birth. Thereby, hopefully reducing the risk of shoulder dystocia.

**P** atient: the patient or, in this case, the problem you are interested in. In our example this would be a pregnant woman with a macrosomic fetus in her third trimester. Or, to be more specific: macrosomia.

**I** ntervention: what intervention are you interested in? For macrosomia this is an induction of labor.

**C** ontrol: what would you like to compare the intervention with? Most of the time this will be the normal general practice. If the intervention is medication, the control could be a placebo. In our case this will be watchful waiting until natural labor starts, also called expectant management.

**O** utcome: this is how you are going to measure the effect of your in-


***Figure 1*** *The logo of Cochrane Library. There is an interesting story to be told about the figure (a forest plot/blobbogram) on the left.*
*© Cochrane Collaboration*

tervention. In other words: what are the criteria that must be met to call your intervention successful? We are interested in the risk of a shoulder dystocia so we take the number of cases as our primary outcome.

Now we have defined all the different components of the research question, we can combine them in a research question: "Does induction of labor in pregnant women with a macrosomic fetus in their third trimester reduce the risk of a shoulder dystocia in comparison to expectant management?" The next step is to enter the PICO in a search engine and pray it will come up with some useful results.

### The search

The easiest way is to enter your PICO in the Trip database: www.Tripdatabase.com. Depending on the question you are interested in and the number of results you get, you can further specify the different components of the PICO. The key is to use short yet specific terms for your PICO.

The second method is more complicated. PubMed: www.ncbi.nlm.nih.gov/pubmed is a database most of you are familiar with. PubMed is funded by the US government and is a database containing biomedical literature from life science journals and online books [6]. The main advantage of PubMed is that articles are manually indexed by their employees. These indexes make it possible to use Mesh-terms (medical subject headings), the use of which enables you to search an entire research field using one specific term. One of the disadvantages is that although PubMed is very complete articles are subjected to quality control, not every journal or article is indexed in PubMed. This means you cannot search every journal there is. To learn more about searching on PubMed there are various tutorials. A good tutorial on PubMed can be found here: https://xot.ru.nl/play.php?template_id=198 or here: https://www.nlm.nih.gov/bsd/viewlet/search/subject/subject.htm written by Pubmed. Another e-learning module about PubMed can be found here: http://ru.nl.libguides.com/medbib/portal. Try to search PubMed using the PICO we described earlier. Let us know what answer you can come up with at: www.ramsresearch.nl.

## Cochrane library

Instead of reviewing all the literature yourself, sometimes experts have already done this in a systematic way, thereby, saving you a lot of time. These systematic reviews can be found in the Cochrane library: www.cochranelibrary.com. Did you know the Cochrane logo shows a meta-analysis proving that corticosteroids given to women who are about to give birth prematurely can save the life of the newborn child [7]? The lines and dots describe the results of different trials showing the benefit of corticosteroids and the diamond the effect after combining all results. However, adoption of the treatment among obstetricians was slow. The publication of a systematic review increased the use of this treatment. This simple intervention has probably saved thousands of premature babies. This example shows how powerful a systematic review can be, so it is worth having a look at this website. The major difference with UpToDate is that reviews in UpToDate are not systematic. Literature selection in UpToDate is based upon expert opinion by a group of physician authors, who will use systematic reviews when available.

## Conclusion

We have taken a look at multiple ways to obtain literature and have drawn the following conclusions. Firstly, it can be useful to use Wikipedia, however UpToDate is a more reliable alternative. Additionally, we learned to formulate a question and search information using the PICO method. Finally, we discussed the Cochrane library, which is useful to find systematic reviews instead of having to review all the literature yourself. I hope this article can be of use in your daily practice and be sure to let us know what answers to PICO questions you can came up with at: www.ramsresearch.nl.

*For more interesting websites and a summary of the discussed literature visit www.ramsresearch.nl*

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# Call for Case Reports

**RAMS is a journal for all (bio)medical students in Nijmegen. Not all students have the available resources to conduct large scale research. Since RAMS does not just focus on research papers, case reports can therefore be a useful way to publish in this medical journal.**

The main goal of a case report is to share a valuable clinical lesson, which is perfectly in line with our aim. For a case report, students are asked to describe the symptoms, signs, diagnosis, treatment and follow up of an individual patient. Students are free to choose their own topic, therefore you do not have to discover your own Elephant Man or an entirely new syndrome. Instead, you can focus on unexpected associations between diseases or symptoms, or unexpected events in the course of observing or treating a patient. You could also produce a case report on: findings that shed new light on the possible pathogenesis of a disease or an adverse effect, the unique or rare features of a disease, unique therapeutic approaches, or a positional or quantitative variation of the anatomical structures. Therefore, the topic of a case report does not have to be a rare disease or a rare complication. Consider writing a case report about more common diagnostic or therapeutic challenges that other students might face some day during one of their internships. After all, the most important criteria are that your report is educational and entertaining.

Take into consideration gaining permission from your supervisor and an informed consent from the patient. More detailed information on how to submit a case report can be found on our website.

Submissions can be sent to submit.rams@ru.nl.

RAMS

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We at RAMS like to think that the Radboud Annals of Medical Students, a medical research magazine made by and aimed at medical students, is a prime example of this. We owe our gratitude to the founders and our readers for making our magazine what it is today. The fact that we are about to publish our fourth edition makes us very proud. The followers on Facebook and our attractive website add to that. Just like the medical world, RAMS is continuously developing.

Big plans are being made for the future, some of which are being put into practice this very moment. RAMS now forms a small part of the new curriculum, various new promotional items were and are given to the students and our website now offers vacancies for temporary jobs.

But why stop there?

The dream of the current board is bigger and even more ambitious. Our goal is to make more students aware of RAMS and the chances and possibilities that RAMS has to offer students about publishing and spreading medical knowledge. To make our dream a reality we need your enthusiasm, knowledge and commitment! Go wild, find a research project and publish it in RAMS. Have you written a research article, case report or essay? Submit it! RAMS is the result of a wild idea, who knows what will become of yours!
Take your chance, publish in RAMS and check out our website (www.ramsresearch.nl)

On behalf of the board of RAMS,

**Sophie van Westendorp, Education**

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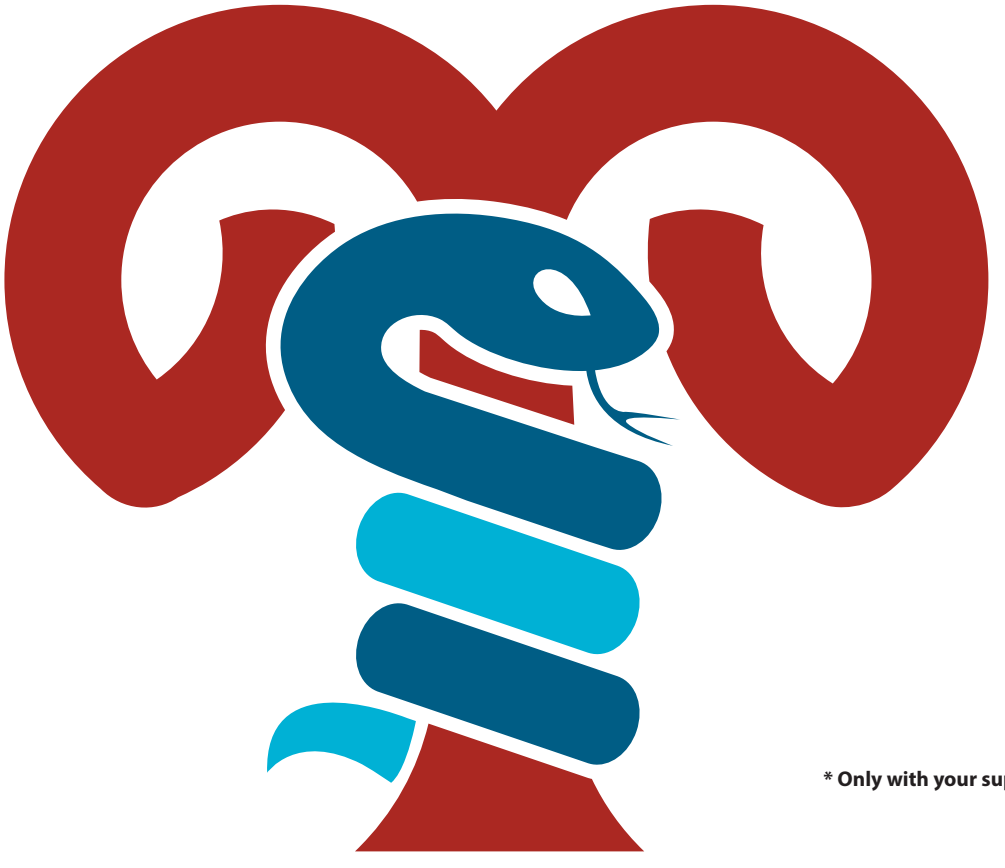

* Only with your supervisor's permission