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The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

In this issue

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Foreword of the editor

Editor-in-Chief: Gábor L. Kovács, MD, PhD, DSc

This themed issue of the eJIFCC on Publication Ethics is based on the lectures of the symposium "PEER REVIEW AND ETHICS IN PUBLICATION IN THE ELECTRONIC AGE" organized by the Communications and Publications Division of the IFCC. The symposium was presented at the IFCC Worldlab Congress in Istanbul in 2014. Organizers of the symposium and thus the guest-editors of this themed issue were Professor Khosrow Adeli (Canada) and Dr. Peter Vervaart (Australia).

Dr. Khosrow Adeli, Ph.D., FCACB, NACB, DABCC is currently a Senior Scientist in the Program in Molecular Structure and Function, Research Institute, The Hospital for Sick Children, University of Toronto. He is also the head and full professor of Clinical Biochemistry at the Hospital for Sick Children and the Departments of Biochemistry, and Laboratory Medicine & Pathobiology at the University of Toronto in Toronto, Canada. He is the Director of Point of Care Testing program at the Hospital for Sick Children in Toronto.

Dr. Adeli is a fellow of the Canadian Academy of Clinical Biochemistry and a diplomate of the American Board of Clinical Biochemistry. He is currently the Editor-in-Chief of the *Critical Reviews in Clinical Laboratory Sciences*. Dr. Adeli served as the Editor-in-Chief of the *Clinical Biochemistry* journal for 7 years (1999-2006). He is an editorial board member of the *Clinical Biochemist Reviews*. He served (2006-2010) as the President of COMACC, the Commission on Accreditation in Clinical Chemistry, a North American organization responsible for accreditation of clinical chemistry training programs in the USA and Canada. He currently serves as the Chair of the Communications and Publications Division of the International Federation of Clinical Chemistry (IFCC).

Dr. Adeli has been actively involved in both basic and clinical laboratory research since 1988 and has published over 250 articles and abstracts in the field of lipid and lipoprotein metabolism, diabetic dyslipidemia, and metabolic disorders. He has received several national and international awards for research excellence including the 2014 Distinguished Service Award of the University of Toronto, the 2011 Canadian Society of Atherosclerosis, Thrombosis and Vascular Biology (CSATVB) Scientific Excellence Award, the 2008 Merck Senior Investigator Award of the Canadian Lipoprotein Conference, the 2006 Canadian Society of Clinical Chemistry National Award for outstanding contributions to clinical chemistry, 2004 Canadian Academy of Clinical Biochemistry National Award, and the 1999 Canadian Society of Clinical Chemistry Research Excellence Award. He has also been active in clinical chemistry research and is the

principal investigator of the CALIPER (Canadian Laboratory Initiative on Pediatric Reference Interval Database) project aimed at the establishment of a laboratory reference interval database for biomarkers of pediatric disease.

Dr. Peter Vervaart, PhD, DipFLMgt, GradCert, PSM, FAACB, FFSc(RCPA) is Principal Scientist in Pathology Services at the Royal Hobart Hospital and has appointments with the University of New South Wales, where he convenes the Molecular Basis of Disease Modules of the Masters in Drug Development, and University of Tasmania. He has a PhD from the University of Melbourne and a Diploma in Frontline Management and Graduate Certificate in Public Sector Management from Swinburne and Flinders Universities respectively. He is a Fellow of the Australasian Association of Clinical Biochemists (AACB) of which he is also President and is a Foundation Fellow of the Faculty of Science of the Royal College of Pathologists of Australasia (RCPA). He is also Publications and Distance Learning Coordinator and Chair of the Internet and e-Learning Committee of the International Federation of Clinical Chemistry (IFCC).

The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

Special eJIFCC issue on peer review and ethics in publication

Guest Editor: Prof. Khosrow Adeli, PhD, FCACB, DABCC

The Communications and Publications Division of the IFCC is pleased to present a special issue of the eJIFCC focused on peer review and ethics in publication. The special issue is based on a recent symposium held during the IFCC WorldLab Congress held in Istanbul, Turkey in June 2014, and includes 4 articles discussing various aspects of scientific peer review and ethics in publication. Peer review is an essential part of the academic writing process and has become an important feature of the scientific community. The process of peer review is used to establish the validity of a body of research or piece of scholarly work, and today, all high impact factor publications are vigorously peer reviewed. Through peer review, an author's work is evaluated by other notable individuals who are experts in that field of study. This process helps maintain the quality of scientific publications, because peer reviewers ensure that the research being presented is original, conclusions are supported by the appropriate experiments, and that the author has not made biased claims based on his/her own personal views. Peer review has become such a foundational pillar of the academic writing process that scientific hypotheses are generally no longer accepted, unless they have been published in a peer reviewed journal.

Despite the benefits of peer review, this process is not perfect, and there have been criticisms regarding the effectiveness of peer review in identifying errors and detecting plagiarism. Additionally, peer-review has been criticized for slowing down the publication process, and for limiting innovative thinking and creative research. With the exponential advancements in online resources that have occurred in recent years, there is now a need to consider the impact of less traditional publications, such as Open Access Journals, and to revisit standards for peer review, publication ethics and scientific writing in general, in light of these electronic developments.

The current issue of eJIFCC is focused on topics related to peer review and ethics in publication. The first article *"Peer review in scientific publications: benefits, critiques & a survival guide"* is a comprehensive guide to the peer review process. Here, Adeli and colleagues summarize the pros and cons of peer review, explain in detail the process of peer review with respect to scientific publications, and give tips for both authors and reviewers to successfully complete this process. In addition, this report highlights the advantages and disadvantages of the different types of peer review (open, double-blind or single-blind), and summarizes new initiatives to improve the peer review process.

The second article, *"Ethics in online publications"* reviews the practice guidelines for ethics in science and in publication, and addresses author responsibilities with respect to publishing. Here, Peter Vervaart puts publication ethics into the context of the electronic age of publishing and open access journals, and highlights issues such as plagiarism and image manipulation in light of the rapidly growing number of journal articles published per year, and the increasing accessibility to scientific information.

The third article, *"Open access publishing in the electronic age"* by Gabor Kovacs, describes the growth of open access publishing as well as the various means of achieving "open access", including open access repositories (the green route to open access), open access journals (the gold route to open access), and platinum open access, which eliminates fees for both the author and the reader. In addition, Kovacs describes copyright licenses for open access, issues with predatory publishing in open access journals, and concludes with the current status of open access publishing in the field of laboratory medicine.

Finally, the article on *"How to write a scientific paper: practical guidelines"* addresses the deficit of appropriate writing experience in new scientific investigators, due to the fact that academic writing skills are no longer a focus of the scientific curriculum. Here, Delvin and colleagues give practical guidelines for writing scientific manuscripts, including a breakdown of the sections needed for a manuscript and what content should be covered under each heading. Furthermore, the authors review the different types of manuscripts, how to target an appropriate journal, and give tips for effective writing.

Taken together, these articles address the current issues with scientific publishing in an electronic era, and provide suggestions for how to publish peer-reviewed articles in high quality open-access journals. The contents of this special issue should benefit both experienced and novice authors of scientific articles, not only in laboratory medicine but also other areas of biological science and medicine.

In this issue: Focusing on Publication Ethics

The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

Peer review in scientific publications: benefits, critiques, & a survival guide

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ABSTRACT

Peer review has been defined as a process of subjecting an author's scholarly work, research or ideas to the scrutiny of others who are experts in the same field. It functions to encourage authors to meet the accepted high standards of their discipline and to control the dissemination of research data to ensure that unwarranted claims, unacceptable interpretations or personal views are not published without prior or expert review. Despite its wide-spread use by most journals, the peer review process has also been widely criticised due to the slowness of the process to publish new findings and due to perceived bias by the editors and/or reviewers. Within the scientific community, peer review has become an essential component of the academic writing process. It helps ensure that papers published in scientific journals answer meaningful research questions and draw accurate conclusions based on professionally executed experimentation. Submission of low quality manuscripts has become increasingly prevalent, and peer review acts as a filter to prevent this work from reaching the scientific community. The major advantage of a peer review process is that peer-reviewed articles provide a trusted form of scientific communication. Since scientific knowledge is cumulative and builds on itself, this trust is particularly important. Despite the positive impacts of peer review, critics argue that the peer review process stifles innovation in experimentation,

and acts as a poor screen against plagiarism. Despite its downfalls, there has not yet been a foolproof system developed to take the place of peer review, however, researchers have been looking into electronic means of improving the peer review process. Unfortunately, the recent explosion in online only/electronic journals has led to mass publication of a large number of scientific articles with little or no peer review. This poses significant risk to advances in scientific knowledge and its future potential. The current article summarizes the peer review process, highlights the pros and cons associated with different types of peer review, and describes new methods for improving peer review.

WHAT IS PEER REVIEW AND WHAT IS ITS PURPOSE?

Peer Review is defined as "a process of subjecting an author's scholarly work, research or ideas to the scrutiny of others who are experts in the same field" (1). Peer review is intended to serve two primary purposes. Firstly, it acts as a filter to ensure that only high quality research is published, especially in reputable journals, by determining the validity, significance and originality of the study. Secondly, peer review is intended to improve the quality of manuscripts that are deemed suitable for publication. Peer reviewers provide suggestions to authors on how to improve the quality of their manuscripts, and also identify any errors that need correcting before publication.

HISTORY OF PEER REVIEW

The concept of peer review was developed long before the scholarly journal. In fact, the peer review process is thought to have been used as a method of evaluating written work since ancient Greece (2). The peer review process was first described by a physician named Ishaq bin Ali al-Rahwi of Syria, who lived from 854-931 CE, in his book *Ethics of the Physician* (2). There, he stated that physicians must take notes describing the state of their patients' medical conditions upon each visit. Following treatment, the notes were scrutinized by a local medical council to determine whether the physician had met the required standards of medical care. If the medical council deemed that the appropriate standards were not met, the physician in question could receive a lawsuit from the maltreated patient (2).

The invention of the printing press in 1453 allowed written documents to be distributed to the general public (3). At this time, it became more important to regulate the quality of the written material that became publicly available, and editing by peers increased in prevalence. In 1620, Francis Bacon wrote the work *Novum Organum*, where he described what eventually became known as the first universal method for generating and assessing new science (3). His work was instrumental in shaping the Scientific Method (3). In 1665, the French *Journal des sçavans* and the English *Philosophical Transactions of the Royal Society* were the first scientific journals to systematically publish research results (4). *Philosophical Transactions of the Royal Society* is thought to be the first journal to formalize the peer review process in 1665 (5), however, it is important to note that peer review was initially introduced to help editors decide which manuscripts to publish in their journals, and at that time it did not serve to ensure the validity of the research (6). It did not take long for the peer review process to evolve, and shortly thereafter papers were distributed to reviewers with the intent of authenticating the integrity of the research study before publication. The Royal Society of Edinburgh adhered to the following peer review process, published in their *Medical Essays and Observations* in 1731: *"Memoirs sent by correspondence are distributed according to the subject matter to those members who*

are most versed in these matters. The report of their identity is not known to the author." (7). The Royal Society of London adopted this review procedure in 1752 and developed the "Committee on Papers" to review manuscripts before they were published in *Philosophical Transactions (*6).

Peer review in the systematized and institutionalized form has developed immensely since the Second World War, at least partly due to the large increase in scientific research during this period (7). It is now used not only to ensure that a scientific manuscript is experimentally and ethically sound, but also to determine which papers sufficiently meet the journal's standards of quality and originality before publication. Peer review is now standard practice by most credible scientific journals, and is an essential part of determining the credibility and quality of work submitted.

IMPACT OF THE PEER REVIEW PROCESS

Peer review has become the foundation of the scholarly publication system because it effectively subjects an author's work to the scrutiny of other experts in the field. Thus, it encourages authors to strive to produce high quality research that will advance the field. Peer review also supports and maintains integrity and authenticity in the advancement of science. A scientific hypothesis or statement is generally not accepted by the academic community unless it has been published in a peer-reviewed journal (8). The Institute for Scientific Information (*ISI*) only considers journals that are peer-reviewed as candidates to receive Impact Factors. Peer review is a well-established process which has been a formal part of scientific communication for over 300 years.

OVERVIEW OF THE PEER REVIEW PROCESS

The peer review process begins when a scientist completes a research study and writes a manuscript that describes the purpose, experimental design, results, and conclusions of the study. The scientist then submits this paper to a suitable journal that specializes in a relevant research field, a step referred to as pre-submission. The editors of the journal will review the paper to ensure that the subject matter is in line with that of the journal, and that it fits with the editorial platform. Very few papers pass this initial evaluation. If the journal editors feel the paper sufficiently meets these requirements and is written by a credible source, they will send the paper to accomplished researchers in the field for a formal peer review. Peer reviewers are also known as referees (this process is summarized in Figure 1). The role of the editor is to select the most appropriate manuscripts for the journal, and to implement and monitor the peer review process. Editors must ensure that peer reviews are conducted fairly, and in an effective and timely manner. They must also ensure that there are no conflicts of interest involved in the peer review process.

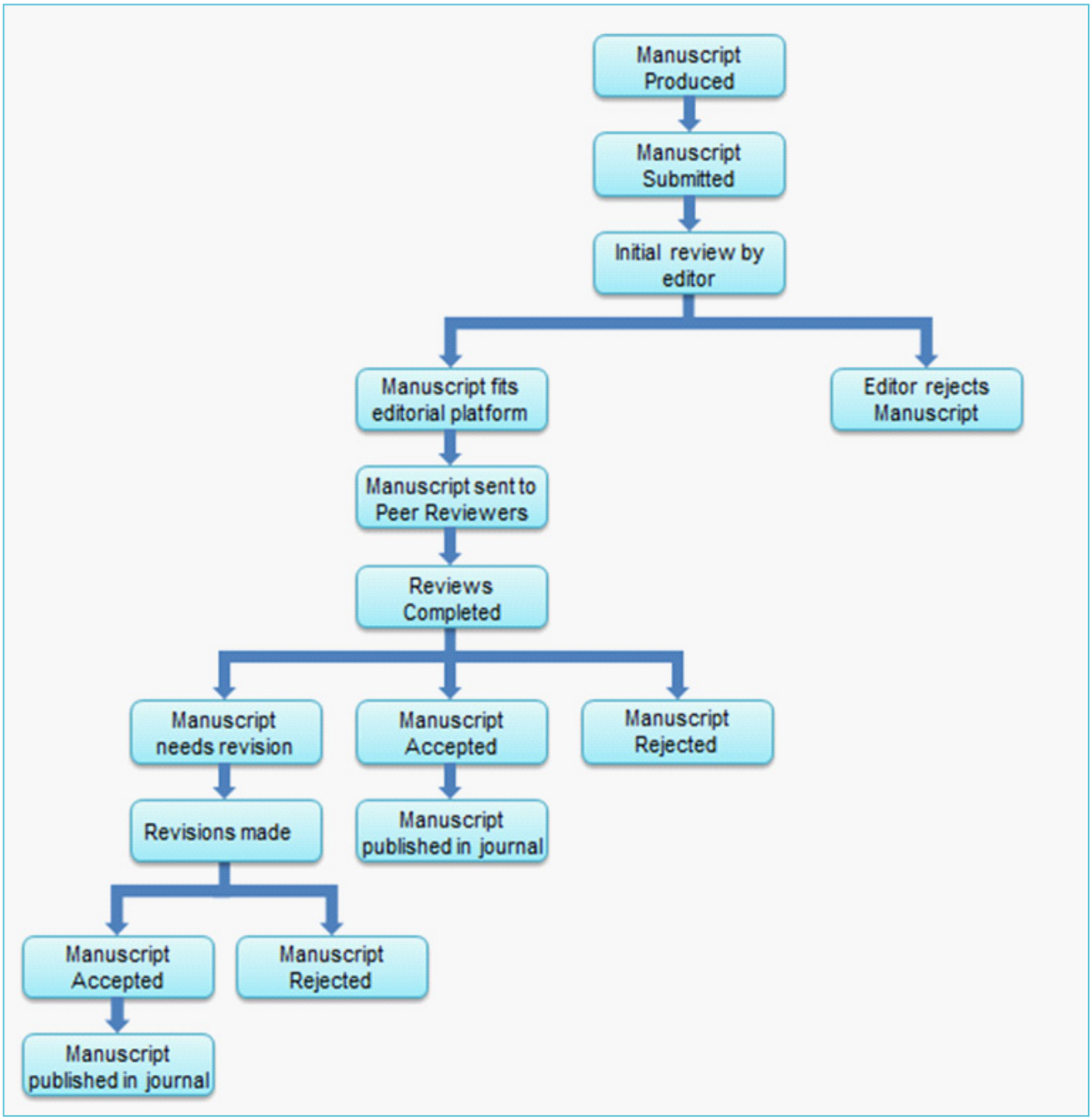
When a reviewer is provided with a paper, he or she reads it carefully and scrutinizes it to evaluate the validity of the science, the quality of the experimental design, and the appropriateness of the methods used. The reviewer also assesses the significance of the research, and judges whether the work will contribute to advancement in the field by evaluating the importance of the findings, and determining the originality of the research. Additionally, reviewers identify any scientific errors and references that are missing or incorrect. Peer reviewers give recommendations to the editor regarding whether the paper should be accepted, rejected, or improved before publication in the journal. The editor will mediate author-referee discussion in order to clarify the priority of certain referee requests, suggest areas that can be strengthened, and overrule reviewer recommendations that are beyond the study's scope (9). If

the paper is accepted, as per suggestion by the peer reviewer, the paper goes into the production stage, where it is tweaked and formatted by the editors, and finally published in the scientific journal. An overview of the review process is presented in Figure 1.

WHO CONDUCTS REVIEWS?

Peer reviews are conducted by scientific experts with specialized knowledge on the content of the manuscript, as well as by scientists with a more general knowledge base. Peer reviewers can be anyone who has competence and

Figure 1 Overview of the review process



expertise in the subject areas that the journal covers. Reviewers can range from young and up-and-coming researchers to old masters in the field. Often, the young reviewers are the most responsive and deliver the best quality reviews, though this is not always the case. On average, a reviewer will conduct approximately eight reviews per year, according to a study on peer review by the Publishing Research Consortium (PRC) (7). Journals will often have a pool of reviewers with diverse backgrounds to allow for many different perspectives. They will also keep a rather large reviewer bank, so that reviewers do not get burnt out, overwhelmed or time constrained from reviewing multiple articles simultaneously.

WHY DO REVIEWERS REVIEW?

Referees are typically not paid to conduct peer reviews and the process takes considerable effort, so the question is raised as to what incentive referees have to review at all. Some feel an academic duty to perform reviews, and are of the mentality that if their peers are expected to review their papers, then they should review the work of their peers as well. Reviewers may also have personal contacts with editors, and may want to assist as much as possible. Others review to keep up-to-date with the latest developments in their field, and reading new scientific papers is an effective way to do so. Some scientists use peer review as an opportunity to advance their own research as it stimulates new ideas and allows them to read about new experimental techniques. Other reviewers are keen on building associations with prestigious journals and editors and becoming part of their community, as sometimes reviewers who show dedication to the journal are later hired as editors. Some scientists see peer review as a chance to become aware of the latest research before their peers, and thus be first to develop new insights from the material. Finally, in terms of career development, peer reviewing can be desirable as it is often noted on one's resume or CV. Many institutions consider a researcher's involvement in peer review when assessing their performance for promotions (11). Peer reviewing can also be an effective way for a scientist to show their superiors that they are committed to their scientific field (5).

ARE REVIEWERS KEEN TO REVIEW?

A 2009 international survey of 4000 peer reviewers conducted by the charity Sense About Science at the British Science Festival at the University of Surrey, found that 90% of reviewers were keen to peer review (12). One third of respondents to the survey said they were happy to review up to five papers per year, and an additional one third of respondents were happy to review up to ten.

HOW LONG DOES IT TAKE TO REVIEW ONE PAPER?

On average, it takes approximately six hours to review one paper (12), however, this number may vary greatly depending on the content of the paper and the nature of the peer reviewer. One in every 100 participants in the "Sense About Science" survey claims to have taken more than 100 hours to review their last paper (12).

HOW TO DETERMINE IF A JOURNAL IS PEER REVIEWED

Ulrichsweb is a directory that provides information on over 300,000 periodicals, including information regarding which journals are peer reviewed (13). After logging into the system using an institutional login (eg. from the University of Toronto), search terms, journal titles or ISSN numbers can be entered into the search bar. The database provides the title, publisher, and country of origin of the journal, and indicates

whether the journal is still actively publishing. The black book symbol (labelled 'refereed') reveals that the journal is peer reviewed.

THE EVALUATION CRITERIA FOR PEER REVIEW OF SCIENTIFIC PAPERS

As previously mentioned, when a reviewer receives a scientific manuscript, he/she will first determine if the subject matter is well suited for the content of the journal. The reviewer will then consider whether the research question is important and original, a process which may be aided by a literature scan of review articles.

Scientific papers submitted for peer review usually follow a specific structure that begins with the title, followed by the abstract, introduction, methodology, results, discussion, conclusions, and references. The title must be descriptive and include the concept and organism investigated, and potentially the variable manipulated and the systems used in the study. The peer reviewer evaluates if the title is descriptive enough, and ensures that it is clear and concise. A study by the National Association of Realtors (NAR) published by the Oxford University Press in 2006 indicated that the title of a manuscript plays a significant role in determining reader interest, as 72% of respondents said they could *usually* judge whether an article will be of interest to them based on the title and the author, while 13% of respondents claimed to *always* be able to do so (14).

The abstract is a summary of the paper, which briefly mentions the background or purpose, methods, key results, and major conclusions of the study. The peer reviewer assesses whether the abstract is sufficiently informative and if the content of the abstract is consistent with the rest of the paper. The NAR study indicated that 40% of respondents could determine whether an article would be of interest to them based on the abstract alone 60-80% of the time, while 32% could judge an article based on the abstract 80-100% of the time (14). This demonstrates that the abstract alone is often used to assess the value of an article.

The introduction of a scientific paper presents the research question in the context of what is already known about the topic, in order to identify why the question being studied is of interest to the scientific community, and what gap in knowledge the study aims to fill (15). The introduction identifies the study's purpose and scope, briefly describes the general methods of investigation, and outlines the hypothesis and predictions (15). The peer reviewer determines whether the introduction provides sufficient background information on the research topic, and ensures that the research question and hypothesis are clearly identifiable.

The methods section describes the experimental procedures, and explains why each experiment was conducted. The methods section also includes the equipment and reagents used in the investigation. The methods section should be detailed enough that it can be used it to repeat the experiment (15). Methods are written in the past tense and in the active voice. The peer reviewer assesses whether the appropriate methods were used to answer the research question, and if they were written with sufficient detail. If information is missing from the methods section, it is the peer reviewer's job to identify what details need to be added.

The results section is where the outcomes of the experiment and trends in the data are explained without judgement, bias or interpretation (15). This section can include statistical tests performed on the data, as well as figures and tables in addition to the text. The peer reviewer ensures that the results are described with sufficient detail, and determines their credibility. Reviewers also confirm that the text is consistent with the information presented in

tables and figures, and that all figures and tables included are important and relevant (15). The peer reviewer will also make sure that table and figure captions are appropriate both contextually and in length, and that tables and figures present the data accurately.

The discussion section is where the data is analyzed. Here, the results are interpreted and related to past studies (15). The discussion describes the meaning and significance of the results in terms of the research question and hypothesis, and states whether the hypothesis was supported or rejected. This section may also provide possible explanations for unusual results and suggestions for future research (15). The discussion should end with a conclusions section that summarizes the major findings of the investigation. The peer reviewer determines whether the discussion is clear and focused, and whether the conclusions are an appropriate interpretation of the results. Reviewers also ensure that the discussion addresses the limitations of the study, any anomalies in the results, the relationship of the study to previous research, and the theoretical implications and practical applications of the study.

The references are found at the end of the paper, and list all of the information sources cited in the text to describe the background, methods, and/or interpret results. Depending on the citation method used, the references are listed in alphabetical order according to author last name, or numbered according to the order in which they appear in the paper. The peer reviewer ensures that references are used appropriately, cited accurately, formatted correctly, and that none are missing.

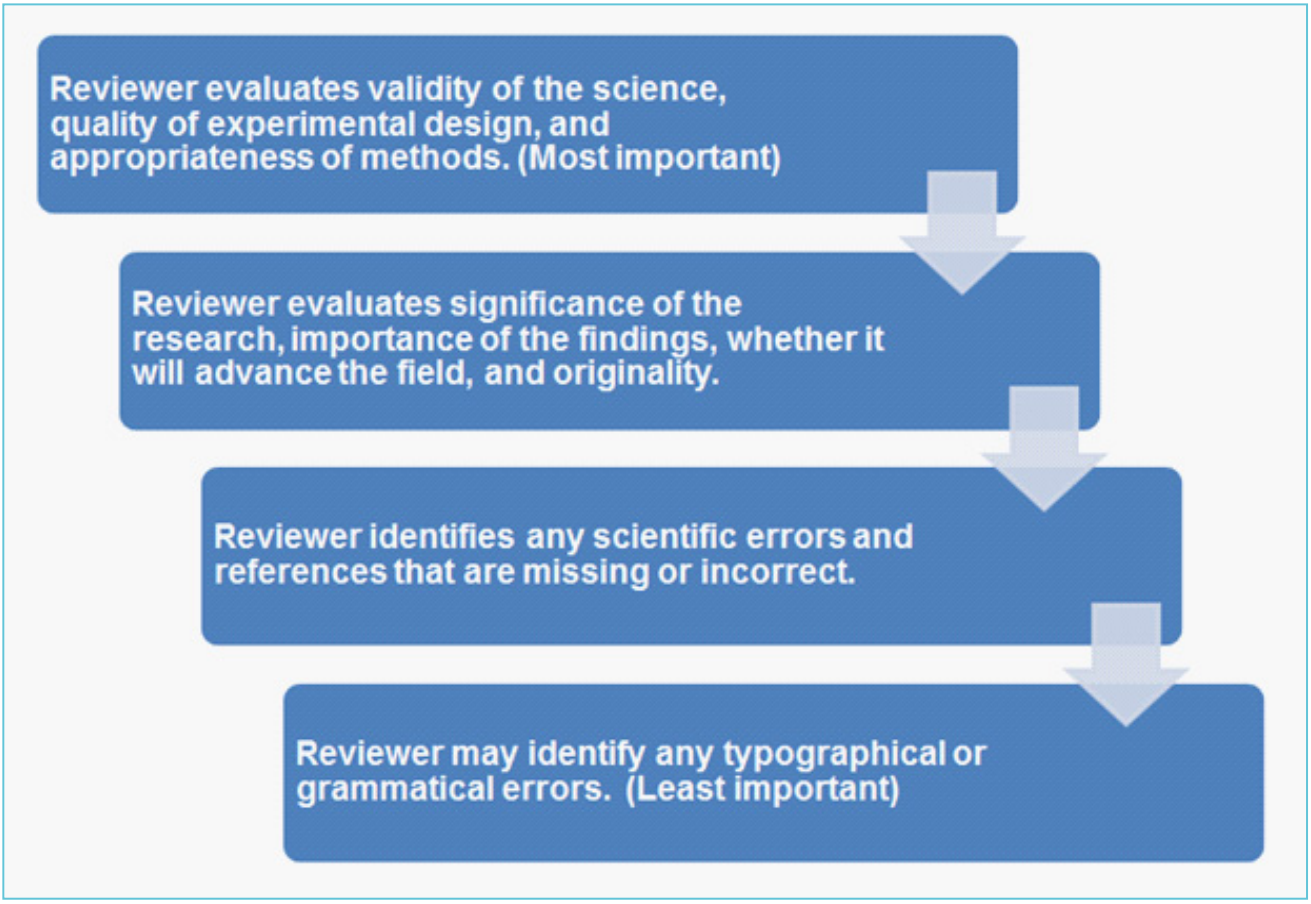
Finally, the peer reviewer determines whether the paper is clearly written and if the content seems logical. After thoroughly reading through the entire manuscript, they determine whether it meets the journal's standards for publication, and whether it falls within the top 25% of papers in its field (16) to determine priority for publication. An overview of what a peer reviewer looks for when evaluating a manuscript, in order of importance, is presented in Figure 2.

To increase the chance of success in the peer review process, the author must ensure that the paper fully complies with the journal guidelines before submission. The author must also be open to criticism and suggested revisions, and learn from mistakes made in previous submissions.

ADVANTAGES AND DISADVANTAGES OF THE DIFFERENT TYPES OF PEER REVIEW

The peer review process is generally conducted in one of three ways: open review, single-blind review, or double-blind review. In an open review, both the author of the paper and the peer reviewer know one another's identity. Alternatively, in single-blind review, the reviewer's identity is kept private, but the author's identity is revealed to the reviewer. In double-blind review, the identities of both the reviewer and author are kept anonymous. Open peer review is advantageous in that it prevents the reviewer from leaving malicious comments, being careless, or procrastinating completion of the review (2). It encourages reviewers to be open and honest without being disrespectful. Open reviewing also discourages plagiarism amongst authors (2). On the other hand, open peer review can also prevent reviewers from being honest for fear of developing bad rapport with the author. The reviewer may withhold or tone down their criticisms in order to be polite (2). This is especially true when younger reviewers are given a more esteemed author's work, in which case the reviewer may be hesitant to provide criticism for fear that it will damper their relationship with a superior (2). According to the Sense About Science survey, editors

Figure 2 How a peer review evaluates a manuscript



find that completely open reviewing decreases the number of people willing to participate, and leads to reviews of little value (12). In the aforementioned study by the PRC, only 23% of authors surveyed had experience with open peer review (7).

Single-blind peer review is by far the most common. In the PRC study, 85% of authors surveyed had experience with single-blind peer review (7). This method is advantageous as the reviewer is more likely to provide honest feedback when their identity is concealed (2). This allows the reviewer to make independent decisions without the influence of the author (2). The main disadvantage of reviewer anonymity, however, is that reviewers who receive manuscripts on subjects similar to their own research may be tempted to delay completing the review in order to publish their own data first (2).

Double-blind peer review is advantageous as it prevents the reviewer from being biased against the author based on their country of origin or previous work (2). This allows the paper to be judged based on the quality of the content, rather than the reputation of the author. The Sense About Science survey indicates that 76% of researchers think double-blind peer review is a good idea (12), and the PRC survey indicates that 45% of authors have had experience with double-blind peer review (7). The disadvantage of double-blind peer review is that, especially in niche areas of research, it can sometimes be easy for the reviewer to determine the identity of the author based on

writing style, subject matter or self-citation, and thus, impart bias (2).

Masking the author's identity from peer reviewers, as is the case in double-blind review, is generally thought to minimize bias and maintain review quality. A study by Justice *et al*. in 1998 investigated whether masking author identity affected the quality of the review (17). One hundred and eighteen manuscripts were randomized; 26 were peer reviewed as normal, and 92 were moved into the 'intervention' arm, where editor quality assessments were completed for 77 manuscripts and author quality assessments were completed for 40 manuscripts (17). There was no perceived difference in quality between the masked and unmasked reviews. Additionally, the masking itself was often unsuccessful, especially with well-known authors (17). However, a previous study conducted by McNutt *et al*. had different results (18). In this case, blinding was successful 73% of the time, and they found that when author identity was masked, the quality of review was slightly higher (18). Although Justice *et al*. argued that this difference was too small to be consequential, their study targeted only biomedical journals, and the results cannot be generalized to journals of a different subject matter (17). Additionally, there were problems masking the identities of well-known authors, introducing a flaw in the methods. Regardless, Justice *et al*. concluded that masking author identity from reviewers may not improve review quality (17).

In addition to open, single-blind and double-blind peer review, there are two experimental forms of peer review. In some cases, following publication, papers may be subjected to post-publication peer review. As many papers are now published online, the scientific community has the opportunity to comment on these papers, engage in online discussions and post a formal review. For example, online publishers PLOS and BioMed Central have enabled scientists to post comments on published papers if they are registered users of the site (10). *Philica* is another journal launched with this experimental form of peer review. Only 8% of authors surveyed in the PRC study had experience with post-publication review (7). Another experimental form of peer review called Dynamic Peer Review has also emerged. Dynamic peer review is conducted on websites such as Naboj, which allow scientists to conduct peer reviews on articles in the preprint media (19). The peer review is conducted on repositories and is a continuous process, which allows the public to see both the article and the reviews as the article is being developed (19). Dynamic peer review helps prevent plagiarism as the scientific community will already be familiar with the work before the peer reviewed version appears in print (19). Dynamic review also reduces the time lag between manuscript submission and publishing. An example of a preprint server is the 'arXiv' developed by Paul Ginsparg in 1991, which is used primarily by physicists (19). These alternative forms of peer review are still unestablished and experimental. Traditional peer review is time-tested and still highly utilized. All methods of peer review have their advantages and deficiencies, and all are prone to error.

PEER REVIEW OF OPEN ACCESS JOURNALS

Open access (OA) journals are becoming increasingly popular as they allow the potential for widespread distribution of publications in a timely manner (20). Nevertheless, there can be issues regarding the peer review process of open access journals. In a study published in Science in 2013, John Bohannon submitted 304 slightly different versions of a fictional scientific paper (written by a fake author, working out of a non-existent institution) to a selected group of OA journals. This study was performed in order to determine whether papers submitted to OA journals are properly reviewed

before publication in comparison to subscription-based journals. The journals in this study were selected from the Directory of Open Access Journals (DOAJ) and Biall's List, a list of journals which are potentially predatory, and all required a fee for publishing (21). Of the 304 journals, 157 accepted a fake paper, suggesting that acceptance was based on financial interest rather than the quality of article itself, while 98 journals promptly rejected the fakes (21). Although this study highlights useful information on the problems associated with lower quality publishers that do not have an effective peer review system in place, the article also generalizes the study results to all OA journals, which can be detrimental to the general perception of OA journals. There were two limitations of the study that made it impossible to accurately determine the relationship between peer review and OA journals: 1) there was no control group (subscription-based journals), and 2) the fake papers were sent to a non-randomized selection of journals, resulting in bias.

JOURNAL ACCEPTANCE RATES

Based on a recent survey, the average acceptance rate for papers submitted to scientific journals is about 50% (7). Twenty percent of the submitted manuscripts that are not accepted are rejected prior to review, and 30% are rejected following review (7). Of the 50% accepted, 41% are accepted with the condition of revision, while only 9% are accepted without the request for revision (7).

SATISFACTION WITH THE PEER REVIEW SYSTEM

Based on a recent survey by the PRC, 64% of academics are satisfied with the current system of peer review, and only 12% claimed to be 'dissatisfied' (7). The large majority, 85%, agreed with the statement that *'scientific communication is greatly helped by peer review'* (7). There was a similarly high level of support (83%) for the idea that peer review *'provides control in scientific communication'* (7).

HOW TO PEER REVIEW EFFECTIVELY

The following are ten tips on how to be an effective peer reviewer as indicated by Brian Lucey, an expert on the subject (22):

1) Be professional

Peer review is a mutual responsibility among fellow scientists, and scientists are expected, as part of the academic community, to take part in peer review. If one is to expect others to review their work, they should commit to reviewing the work of others as well, and put effort into it.

2) Be pleasant

If the paper is of low quality, suggest that it be rejected, but do not leave ad hominem comments. There is no benefit to being ruthless.

3) Read the invite

When emailing a scientist to ask them to conduct a peer review, the majority of journals will provide a link to either accept or reject. Do not respond to the email, respond to the link.

4) Be helpful

Suggest how the authors can overcome the shortcomings in their paper. A review should guide the author on what is good and what needs work from the reviewer's perspective.

5) Be scientific

The peer reviewer plays the role of a scientific peer, not an editor for proofreading or decision-making. Don't fill a review with comments on editorial and typographic issues. Instead, focus on adding value with scientific knowledge and commenting on the credibility of the research

conducted and conclusions drawn. If the paper has a lot of typographical errors, suggest that it be professionally proof edited as part of the review.

6) Be timely

Stick to the timeline given when conducting a peer review. Editors track who is reviewing what and when and will know if someone is late on completing a review. It is important to be timely both out of respect for the journal and the author, as well as to not develop a reputation of being late for review deadlines.

7) Be realistic

The peer reviewer must be realistic about the work presented, the changes they suggest and their role. Peer reviewers may set the bar too high for the paper they are editing by proposing changes that are too ambitious and editors must override them.

8) Be empathetic

Ensure that the review is scientific, helpful and courteous. Be sensitive and respectful with word choice and tone in a review.

9) Be open

Remember that both specialists and generalists can provide valuable insight when peer reviewing. Editors will try to get both specialised and general reviewers for any particular paper to allow for different perspectives. If someone is asked to review, the editor has determined they have a valid and useful role to play, even if the paper is not in their area of expertise.

10) Be organised

A review requires structure and logical flow. A reviewer should proofread their review before submitting it for structural, grammatical and spelling errors as well as for clarity. Most publishers provide short guides on structuring a peer review on their website. Begin with an overview of the proposed improvements; then provide feedback on the paper structure, the quality of data sources and methods of investigation used, the logical flow of argument, and the validity of conclusions drawn. Then provide feedback on style, voice and lexical concerns, with suggestions on how to improve.

In addition, the American Physiology Society (APS) recommends in its Peer Review 101 Handout that peer reviewers should put themselves in both the editor's and author's shoes to ensure that they provide what both the editor and the author need and expect (11). To please the editor, the reviewer should ensure that the peer review is completed on time, and that it provides clear explanations to back up recommendations. To be helpful to the author, the reviewer must ensure that their feedback is constructive. It is suggested that the reviewer take time to think about the paper; they should read it once, wait at least a day, and then re-read it before writing the review (11). The APS also suggests that Graduate students and researchers pay attention to how peer reviewers edit their work, as well as to what edits they find helpful, in order to learn how to peer review effectively (11). Additionally, it is suggested that Graduate students practice reviewing by editing their peers' papers and asking a faculty member for feedback on their efforts. It is recommended that young scientists offer to peer review as often as possible in order to become skilled at the process (11). The majority of students, fellows and trainees do not get formal training in peer review, but rather learn by observing their mentors. According to the APS, one acquires experience through networking and referrals, and should therefore try to strengthen relationships with journal editors by offering to review manuscripts (11). The APS also suggests that experienced reviewers provide constructive feedback

to students and junior colleagues on their peer review efforts, and encourages them to peer review to demonstrate the importance of this process in improving science (11).

The peer reviewer should only comment on areas of the manuscript that they are knowledgeable about (23). If there is any section of the manuscript they feel they are not qualified to review, they should mention this in their comments and not provide further feedback on that section. The peer reviewer is not permitted to share any part of the manuscript with a colleague (even if they may be more knowledgeable in the subject matter) without first obtaining permission from the editor (23). If a peer reviewer comes across something they are unsure of in the paper, they can consult the literature to try and gain insight. It is important for scientists to remember that if a paper can be improved by the expertise of one of their colleagues, the journal must be informed of the colleague's help, and approval must be obtained for their colleague to read the protected document. Additionally, the colleague must be identified in the confidential comments to the editor, in order to ensure that he/she is appropriately credited for any contributions (23). It is the job of the reviewer to make sure that the colleague assisting is aware of the confidentiality of the peer review process (23). Once the review is complete, the manuscript must be destroyed and cannot be saved electronically by the reviewers (23).

COMMON ERRORS IN SCIENTIFIC PAPERS

When performing a peer review, there are some common scientific errors to look out for. Most of these errors are violations of logic and common sense: these may include contradicting statements, unwarranted conclusions, suggestion of causation when there is only support for correlation, inappropriate extrapolation, circular reasoning, or pursuit of a trivial question (24). It is also common for authors to suggest that two variables are different because the effects of one variable are statistically significant while the effects of the other variable are not, rather than directly comparing the two variables (24). Authors sometimes oversee a confounding variable and do not control for it, or forget to include important details on how their experiments were controlled or the physical state of the organisms studied (24). Another common fault is the author's failure to define terms or use words with precision, as these practices can mislead readers (24). Jargon and/or misused terms can be a serious problem in papers. Inaccurate statements about specific citations are also a common occurrence (24). Additionally, many studies produce knowledge that can be applied to areas of science outside the scope of the original study, therefore it is better for reviewers to look at the novelty of the idea, conclusions, data, and methodology, rather than scrutinize whether or not the paper answered the specific question at hand (24). Although it is important to recognize these points, when performing a review it is generally better practice for the peer reviewer to not focus on a checklist of things that could be wrong, but rather carefully identify the problems specific to each paper and continuously ask themselves if anything is missing (24). An extremely detailed description of how to conduct peer review effectively is presented in the paper *How I Review an Original Scientific Article* written by Frederic G. Hoppin, Jr. It can be accessed through the American Physiological Society website under the Peer Review Resources section.

CRITICISM OF PEER REVIEW

A major criticism of peer review is that there is little evidence that the process actually works, that it is actually an effective screen for good quality scientific work, and that it actually

improves the quality of scientific literature. As a 2002 study published in the *Journal of the American Medical Association* concluded, *'Editorial peer review, although widely used, is largely untested and its effects are uncertain'* (25). Critics also argue that peer review is not effective at detecting errors. Highlighting this point, an experiment by Godlee *et al*. published in the *British Medical Journal* (BMJ) inserted eight deliberate errors into a paper that was nearly ready for publication, and then sent the paper to 420 potential reviewers (7). Of the 420 reviewers that received the paper, 221 (53%) responded, the average number of errors spotted by reviewers was two, no reviewer spotted more than five errors, and 35 reviewers (16%) did not spot any.

Another criticism of peer review is that the process is not conducted thoroughly by scientific conferences with the goal of obtaining large numbers of submitted papers. Such conferences often accept any paper sent in, regardless of its credibility or the prevalence of errors, because the more papers they accept, the more money they can make from author registration fees (26). This misconduct was exposed in 2014 by three MIT graduate students by the names of Jeremy Stribling, Dan Aguayo and Maxwell Krohn, who developed a simple computer program called SCIgen that generates nonsense papers and presents them as scientific papers (26). Subsequently, a nonsense SCIgen paper submitted to a conference was promptly accepted. *Nature* recently reported that French researcher Cyril Labbé discovered that sixteen SCIgen nonsense papers had been used by the German academic publisher Springer (26). Over 100 nonsense papers generated by SCIgen were published by the US Institute of Electrical and Electronic Engineers (IEEE) (26). Both organisations have been working to remove the papers. Labbé developed a program to detect SCIgen papers and has made it freely available to ensure publishers and conference organizers do not accept nonsense work in the future. It is available at this link: http://scigendetection.imag.fr/main.php (26).

Additionally, peer review is often criticized for being unable to accurately detect plagiarism. However, many believe that detecting plagiarism cannot practically be included as a component of peer review. As explained by Alice Tuff, development manager at Sense About Science, *'The vast majority of authors and reviewers think peer review should detect plagiarism (81%) but only a minority (38%) think it is capable. The academic time involved in detecting plagiarism through peer review would cause the system to grind to a halt*' (27). Publishing house Elsevier began developing electronic plagiarism tools with the help of journal editors in 2009 to help improve this issue (27).

It has also been argued that peer review has lowered research quality by limiting creativity amongst researchers. Proponents of this view claim that peer review has repressed scientists from pursuing innovative research ideas and bold research questions that have the potential to make major advances and paradigm shifts in the field, as they believe that this work will likely be rejected by their peers upon review (28). Indeed, in some cases peer review may result in rejection of innovative research, as some studies may not seem particularly strong initially, yet may be capable of yielding very interesting and useful developments when examined under different circumstances, or in the light of new information (28). Scientists that do not believe in peer review argue that the process stifles the development of ingenious ideas, and thus the release of fresh knowledge and new developments into the scientific community.

Another issue that peer review is criticized for, is that there are a limited number of people that are competent to conduct peer review

compared to the vast number of papers that need reviewing. An enormous number of papers published (1.3 million papers in 23,750 journals in 2006), but the number of competent peer reviewers available could not have reviewed them all (29). Thus, people who lack the required expertise to analyze the quality of a research paper are conducting reviews, and weak papers are being accepted as a result. It is now possible to publish any paper in an obscure journal that claims to be peer-reviewed, though the paper or journal itself could be substandard (29). On a similar note, the US National Library of Medicine indexes 39 journals that specialize in alternative medicine, and though they all identify themselves as "peer-reviewed", they rarely publish any high quality research (29). This highlights the fact that peer review of more controversial or specialized work is typically performed by people who are interested and hold similar views or opinions as the author, which can cause bias in their review. For instance, a paper on homeopathy is likely to be reviewed by fellow practicing homeopaths, and thus is likely to be accepted as credible, though other scientists may find the paper to be nonsense (29). In some cases, papers are initially published, but their credibility is challenged at a later date and they are subsequently retracted. Retraction Watch is a website dedicated to revealing papers that have been retracted after publishing, potentially due to improper peer review (30).

Additionally, despite its many positive outcomes, peer review is also criticized for being a delay to the dissemination of new knowledge into the scientific community, and as an unpaid-activity that takes scientists' time away from activities that they would otherwise prioritize, such as research and teaching, for which they are paid (31). As described by Eva Amsen, Outreach Director for F1000Research, peer review was originally developed as a means of helping editors choose which papers to publish when journals had to limit the number of papers they could print in one issue (32). However, nowadays most journals are available online, either exclusively or in addition to print, and many journals have very limited printing runs (32). Since there are no longer page limits to journals, any good work can and should be published. Consequently, being selective for the purpose of saving space in a journal is no longer a valid excuse that peer reviewers can use to reject a paper (32). However, some reviewers have used this excuse when they have personal ulterior motives, such as getting their own research published first.

RECENT INITIATIVES TOWARDS IMPROVING PEER REVIEW

F1000Research was launched in January 2013 by Faculty of 1000 as an open access journal that immediately publishes papers (after an initial check to ensure that the paper is in fact produced by a scientist and has not been plagiarised), and then conducts transparent post-publication peer review (32). F1000Research aims to prevent delays in new science reaching the academic community that are caused by prolonged publication times (32). It also aims to make peer reviewing more fair by eliminating any anonymity, which prevents reviewers from delaying the completion of a review so they can publish their own similar work first (32). F1000Research offers completely open peer review, where everything is published, including the name of the reviewers, their review reports, and the editorial decision letters (32).

PeerJ was founded by Jason Hoyt and Peter Binfield in June 2012 as an open access, peer reviewed scholarly journal for the Biological and Medical Sciences (33). PeerJ selects articles to publish based only on scientific and methodological soundness, not on subjective determinants

of 'impact,' 'novelty' or 'interest' (34). It works on a "lifetime publishing plan" model which charges scientists for publishing plans that give them lifetime rights to publish with PeerJ, rather than charging them per publication (34). PeerJ also encourages open peer review, and authors are given the option to post the full peer review history of their submission with their published article (34). PeerJ also offers a pre-print review service called PeerJ Pre-prints, in which paper drafts are reviewed before being sent to PeerJ to publish (34).

Rubriq is an independent peer review service designed by Shashi Mudunuri and Keith Collier to improve the peer review system (35). Rubriq is intended to decrease redundancy in the peer review process so that the time lost in redundant reviewing can be put back into research (35). According to Keith Collier, over 15 million hours are lost each year to redundant peer review, as papers get rejected from one journal and are subsequently submitted to a less prestigious journal where they are reviewed again (35). Authors often have to submit their manuscript to multiple journals, and are often rejected multiple times before they find the right match. This process could take months or even years (35). Rubriq makes peer review portable in order to help authors choose the journal that is best suited for their manuscript from the beginning, thus reducing the time before their paper is published (35). Rubriq operates under an author-pay model, in which the author pays a fee and their manuscript undergoes double-blind peer review by three expert academic reviewers using a standardized scorecard (35). The majority of the author's fee goes towards a reviewer honorarium (35). The papers are also screened for plagiarism using iThenticate (35). Once the manuscript has been reviewed by the three experts, the most appropriate journal for submission is determined based on the topic and quality of the paper (35). The paper is returned to the author in 1-2 weeks with the Rubriq Report (35). The author can then submit their paper to the suggested journal with the Rubriq Report attached. The Rubriq Report will give the journal editors a much stronger incentive to consider the paper as it shows that three experts have recommended the paper to them (35). Rubriq also has its benefits for reviewers; the Rubriq scorecard gives structure to the peer review process, and thus makes it consistent and efficient, which decreases time and stress for the reviewer. Reviewers also receive feedback on their reviews and most significantly, they are compensated for their time (35). Journals also benefit, as they receive pre-screened papers, reducing the number of papers sent to their own reviewers, which often end up rejected (35). This can reduce reviewer fatigue, and allow only higher-quality articles to be sent to their peer reviewers (35).

According to Eva Amsen, peer review and scientific publishing are moving in a new direction, in which all papers will be posted online, and a post-publication peer review will take place that is independent of specific journal criteria and solely focused on improving paper quality (32). Journals will then choose papers that they find relevant based on the peer reviews and publish those papers as a collection (32). In this process, peer review and individual journals are uncoupled (32). In Keith Collier's opinion, post-publication peer review is likely to become more prevalent as a complement to pre-publication peer review, but not as a replacement (35). Post-publication peer review will not serve to identify errors and fraud but will provide an additional measurement of impact (35). Collier also believes that as journals and publishers consolidate into larger systems, there will be stronger potential for "cascading" and shared peer review (35).

CONCLUDING REMARKS

Peer review has become fundamental in assisting editors in selecting credible, high quality, novel and interesting research papers to publish in scientific journals and to ensure the correction of any errors or issues present in submitted papers. Though the peer review process still has some flaws and deficiencies, a more suitable screening method for scientific papers has not yet been proposed or developed. Researchers have begun and must continue to look for means of addressing the current issues with peer review to ensure that it is a full-proof system that ensures only quality research papers are released into the scientific community.

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In this issue: Focusing on Publication Ethics

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eJIFCC

Ethics in online publications

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ABSTRACT

Journals have been publishing the results of scientific investigations since the founding of *Philosophical Transactions* in 1665. Since then we have witnessed a massive expansion in the number of journals to the point that there are now approximately 28,000 active, peer reviewed journals collectively publishing more than 1.8 million articles per year. Before the mid-1990s, these journals were only available on paper but by the end of the 20th century, most journals had moved to online platforms. Online publication has also served as the impetus for the move to 'open-access' to the information contained in journals. The fact that a publication is 'on-line' and 'open-access' does not negate the responsibility of the author and the publisher to publish in an ethical way. [1]

The document produced by the IFCC Ethics Task Force (TF-E) on publication ethics states that 'Ethics in Science at its broadest level encompasses research ethics, medical ethics, publication ethics, conflicts of interest, ethical responsibilities as educator, plus many other areas.' Thus publication ethics is a continuum from the first step of research design through to the information being read by the reader.

In general terms 'publication ethics' includes the ethical behaviour of the authors in writing and submitting a scientific manuscript to a publisher for the purpose of publication, thus any discussion of publication ethics must include the role of

the authors, referees, publisher and reader and the issues of authorship (and the use of 'ghosts'), plagiarism, duplicate publication (including in different languages), image manipulation (particularly in the era of digitisation), and conflict of interest [2]. To aid the authors, and others involved in the process of publication, a number of resources are now available particularly those from the Committee on Publication Ethics (COPE) [3] and the World Association of Medical Editors (WAME) [4].

More recently the issue of 'publisher ethics' has also been raised, particularly with the sudden increase of what could be termed 'predatory' publishers utilising the open access model to publish low quality articles, which often do not adhere to the guidelines mentioned above, utilising an author-pays model of open-access publishing for their own profit [5].

INTRODUCTION

Journals have been publishing the results of scientific investigations since the founding of *Philosophical Transactions* in 1665. Before the mid-1990s journals were only available on paper but by the end of the 20th century most journals have moved to online platforms (or a mix of both paper and online). Online publication has also served as the impetus for the move to 'open-access' publication defined as unrestricted online access to peer-reviewed scholarly research. However 'on-line' and 'open-access' does not negate the responsibility of the author and the publisher to publish in an ethical way.

OPEN ACCESS

As mentioned in the introduction open access means unrestricted online access to peer-reviewed scholarly research. There are two general types: *Gratis* or *Libre* open access defined by whether the access is completely 'open' or in the case of *Libre* whether there is additional usage rights applied. In most cases of *Libre* open access the usage rights are Creative Commons based meaning that they are public copyright licences allowing the free distribution of an otherwise copyrighted article [6]. There are three forms of open access described: Green, Gold and Platinum. Green open access, sometimes known as self-archiving, involves the article being deposited in an institutional repository which is then accessed while Gold open access involves publishing within a journal where the cost of publishing is levied on the author (or authors representative such as their research institution) for the purpose of allowing the article to be then open access. The eJIFCC is an example of a Platinum open access journal where there is no charge levied either on the author (or representative) or the reader with the costs being born by either the journal, or by donations [7].

IFCC TASK FORCE-ETHICS

The IFCC has a particular interest in Ethics and during the Executive Board term of 1997-1999, the IFCC established an Ethics Task Force (TF-E) which is currently chaired by Prof David Bruns of the University of Virginia Medical School. The stated aims of the Task Force are as follows:

- To increase awareness among Laboratory Medicine Professionals of ethical issues
- To encourage the practice of Laboratory Medicine to the highest ethical standards
- To develop position papers on appropriate ethics policy issues
- To provide a voice for Laboratory Medicine on ethics policies
- To link Laboratory Medicine, ethics and the public interest

In response in particular to Aim 3 above the Task Force recently produced a position paper entitled 'Ethics in Science: Background and Resources on Publication Ethics'.

ETHICS IN SCIENCE: BACKGROUND AND RESOURCES ON PUBLICATION ETHICS

This position paper published by the Ethics Task Force, and available for download from the IFCC website (www.ifcc.org), was prepared to bring together a set of resources on publication ethics for use in the field of laboratory medicine. The paper provides background information and advice in the following areas:

- **Research ethics**
  - Human experimentation
  - Animal experimentation
- **Data collection**
- **Publication ethics**
  - Author aspects
    - Authorship
    - Plagiarism
    - Duplicate publishing
    - Publishing translations of previous work
    - Image manipulation
    - Conflict of interest
- **Referee aspects**
  - Plagiarism, duplicate publishing or other ethical violations
  - Conflict of interest
- **Readers aspects**
  - Plagiarism, duplicate publishing or other ethical violations
- **Editor aspects**
  - Plagiarism, duplicate publishing or other ethical violations responsibility
  - Conflict of interest
- **Conflict of interest in general**
- **Responsibility as educator**

THE COMMITTEE ON PUBLICATION ETHICS (COPE)

There are a number of other sources of information related to Publication Ethics among which includes the Committee on Publication Ethics (COPE). COPE was established in 1997 by a group of medical journal editors in the UK and now has over 9000 members worldwide and is open to editors of academic journals and others interested in publication ethics. Their website (http://publicationethics.org/) is an excellent resource for those with an interest in Publication Ethics and, in particular, provides guidance on how to handle cases of research and publication misconduct.

THE WORLD ASSOCIATION OF MEDICAL EDITORS (WAME)

Another resource is the World Association of Medical Editors (WAME). WAME is a global association of editors of peer-reviewed medical journals with the aim of improving editorial standards through cooperation and communication. Amongst the resources on its website (http://www.wame.org/) are published documents related to Publication Ethics Policies for Medical Journals.

THE INTERNATIONAL COMMITTEE OF MEDICAL JOURNAL EDITORS (ICMJE)

A third group is the International Committee of Medical Journal Editors (ICMJE) which is a small, closed group of general medical journal editors and representatives of selected related organizations whose primary aim is to improve the quality of medical science and its reporting through publication of the *Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals* [8].

PUBLICATION ETHICS

Publication Ethics is a continuum from the first step of research design through to the information being read by the reader and thus includes the ethical behaviour of the authors in writing and submitting a scientific manuscript to a publisher for the purpose of publication but must also include the role of referees, editors, publishers and even the reader in the process.

RESEARCH MISCONDUCT

By definition Research Misconduct means the Fabrication, Falsification, or Plagiarism in proposing, performing, or reviewing research, or in reporting research results. Fabrication is the making up of data or results and recording or reporting them as if they were real while Falsification is manipulating research materials, equipment, or processes, or changing or omitting data or results such that the research is not accurately represented in the research record. Plagiarism is well defined as the appropriation of another person's ideas, processes, results, or words without giving appropriate credit and will be covered in more detail later in this paper. It is important to be reminded that Research Misconduct is purposeful misconduct and as such does not include honest error or differences of opinion which may occur at time to time in research and which can generally be corrected or outlined at the time of publication.

THE AUTHOR

The author(s) of a paper are obviously the 'primary' participant in the publication process as without them there would not be a publication. As such the author(s) are of particular importance to ensure ethical publication across various issues related to authorship including plagiarism, duplicate publication, image manipulation and conflict of interest.

AUTHORSHIP

The first step in the process should be to decide on what basis Authorship credit should be applied to a particular individual. In general terms authorship should be determined by substantial contribution to the research and writing of the manuscript, participation in the critical drafting and/or revision of the manuscript and final approval of the document for submission to a journal. Of particular concern in the area of authorship is the use of 'Ghost' authors or writers.

The term ghostwriting can cover a myriad of scenarios and uses ranging from political speech writing through to the publishing of celebrity memoirs and as such can have a varying degree of 'ethics' associated with it. The major issue with medical ghostwriting is the payment of ghostwriters by pharmaceutical companies to produce papers and then the recruitment of other scientists or physicians to attach their names to these papers before they are published in medical or scientific journals. In response to this issue a number of professional medical writers associations have been formed (e.g. European Medical Writers Association) with the aim of ensuring that professional medical writers are acknowledged for their contribution, if not as an author then as a professional writer, and that they carry out this role in an ethical and responsible manner [9]. As a consequence organisations such as the World Association of Medical Editors and the British Medical Journal now accept this as a legitimate practice [10]

AUTHOR RESPONSIBILITIES

There are a number of General Rules which should be followed by Authors when writing and publishing in the medical literature. The first is to ensure that the work they are publishing is for new and original research. Secondly, all listed Authors must be aware of the

submission and must agree with the content and support the submission otherwise there could potentially be embarrassment all round if an author's name pops up on PubMed or similar against a manuscript of which they have no knowledge! The authors must also agree that the manuscript can be examined by anonymous reviewers as anonymous peer review is critical to the publishing process (and will be discussed more fully later). They must also provide copies of related work submitted or published elsewhere as a protection against the possibility of being accused of duplicate publications (also to be discussed later). They must obtain copyright permission if figures/tables need to be reproduced and more importantly must wait until such permission is obtained before going ahead with the publication process. Finally, the authors need to include proper, multiple if necessary, affiliations on the paper so that the reader is able to discern any potential conflicts of interest, and contact the authors to ask questions etc. if necessary.

PLAGIARISM

The increasing availability of scientific literature on the World Wide Web has proven to be a double-edged sword by allowing plagiarism to be more easily committed by 'cut and paste' of content published on the web but at the same time enabling its simple detection through manual on-line review and/or the use of automated comparative software such as free software 'Plagiarism Checker' or commercial software such as Turnitin. The automated process generally involves the use of a form of document 'fingerprinting' whereby multiple digests of a document are compared to a reference library of document 'fingerprints' and, using a complex algorithm, a 'similarity index' is calculated. This index, and offending passages, can then be reviewed and a final assessment made [11]. It is good practice when plagiarism is detected that, as well as the authors(s), the Editor of the journal where the offending article appeared should be contacted to request retraction as well as the Publisher of the journal in which the original authors article appeared to advise breach of copyright.

IMAGE MANIPULATION

In the era of digital images and the use of software such as Photoshop, image manipulation has become an increasingly concerning ethical issue in publications. More recently this has led to the development of the six Clinical and Laboratory Images in Publications (CLIP) Principles:

1. Report the details of the subject of the image

The author should tell readers what they are looking at and what they should be looking for in particular in support of the claims they make associated with the image in the text of the article.

2. Report the details of the acquisition of the image

The authors also need to explain how the image was acquired including any equipment, special techniques, etc. used in the acquisition of the image.

3. Report the details of the selection of the image

The authors should explain why a particular image was selected, whether it was an image intrinsic to the research or whether it was from an outside source and whether it was indicative of the study or an 'extreme' example.

4. Report the details of any modifications of the image

The authors also need to disclose any manipulation of the image, for any purpose, such as enhancements, modifications or processing of the

image. Where possible the full and unaltered image should be published however there may be occasions where for reasons of clarity to the reader the image may be manipulated which is allowed but only where this manipulation is disclosed to the reader.

5. Report the important details of the image itself

Authors should give as much information as possible to allow the reader to interpret the image and relate it contextually to the information provided in the text. This could include the use of annotation tools such as arrows, circles, etc. and information about magnifications etc.

6. Report the details of the analysis or interpretation and the implications of the image

Finally the authors should include the details of any measurements and or analysis of the image which has occurred and how those measurements or analysis have added to the interpretation and findings of the paper [12].

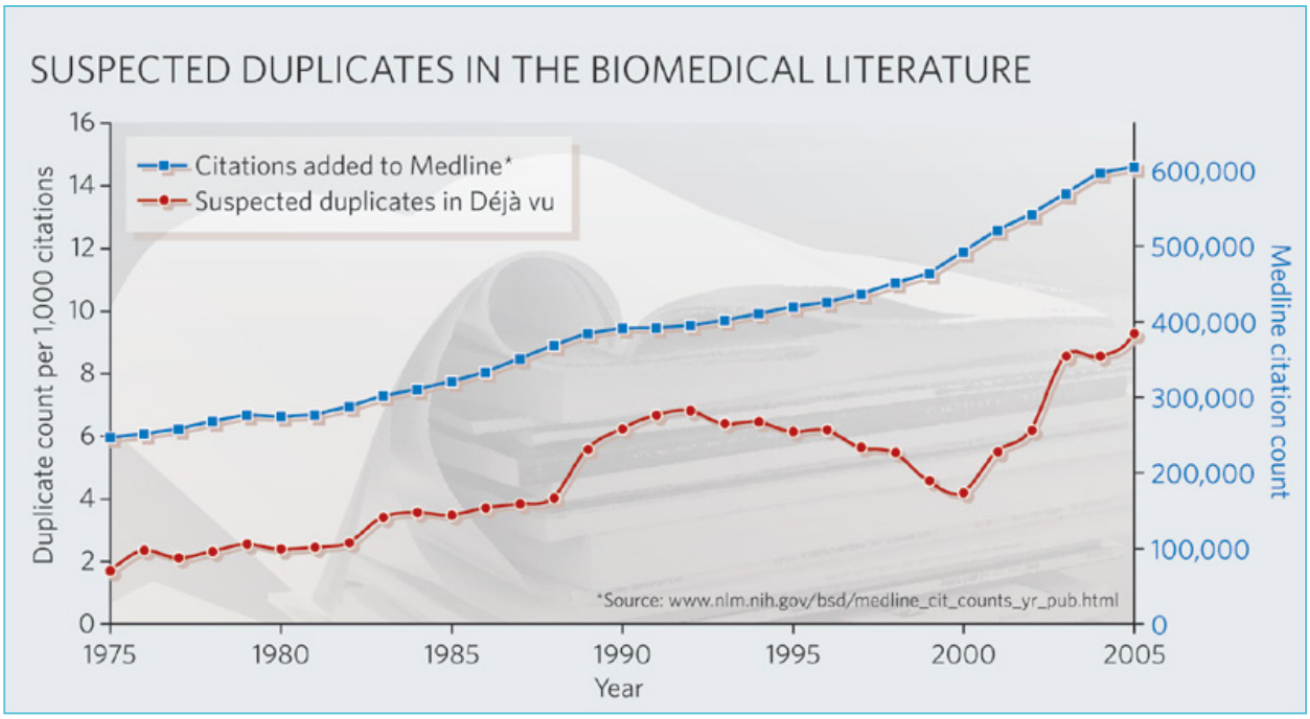
DUPLICATE PUBLICATIONS

Duplicate publication is becoming an increasingly important issue, particularly in the era of globalisation of research and availability of foreign language journals. In general terms, author(s) should avoid publication in duplicate journals and this should include foreign language journals. The size of the problem is indicated in figure 1 which shows the increase in duplicate journals detected by the software deja vue with time.

CONFLICT OF INTEREST

Conflicts of Interest arise when authors, reviewers, or editors have interests that are not fully apparent and that may influence their judgments on what is to be published and if revealed would make a reasonable reader feel misled or deceived by their conduct. Such conflicts can arise from relationships, allegiances, or hostilities to particular groups, organizations, or interests and can be public and/or private (i.e. not obvious from knowledge of the individuals involved and/or

Figure 1 Suspected duplicate publications in the medical literature [13]



associated with a significant other). Real or potential conflicts should be declared as soon as possible during the process to alleviate any concerns particularly as it is difficult to resolve such conflicts of interest after the event and as such the conflict will impact on the perception of the publication whether the conflict was real or not.

REFEREES

Referees or peer reviewers are an important part of the peer-reviewed publication process and as such many of the ethical considerations associated with the author(s) should also apply to the referee, in particular issues around conflict of interest discussed above. Therefore the referee should peer-review with impartiality and confidentiality. They must not contact the author directly and should disclose any potential or real conflicts of interest and they should destroy any manuscripts once the peer review process is complete.

EDITORS

Editors are also central to the ethical publication process and their importance is evidenced by the fact that there are at least two organisations which focus on providing guidelines and advice to Editors involved in the publication process (WAME and ICMJE). In addition COPE states that Editors should be accountable for everything published in their journals. ICMJE states that Editors also have the responsibility of following up complaints about specific articles published in their journal and that Editors should avoid selecting external peer reviewers with obvious potential conflicts of interest. Editors should also have the independence and responsibility to retract papers following a breach of ethics.

PUBLISHERS

Publishers have a responsibility to the scientific record to ensure that the journals they publish are as free of publishing ethics violations as they can be. They also need to respect the privacy and rights of researchers and protect the intellectual property and copyright of the authors. As mentioned above publishers also need to foster the editorial independence of the publishing process by granting Editors with the authority and responsibility to retract papers following a breach of ethics without fear nor favour. More recently we have seen a move towards a form of Predatory Publishing which is worrying many in the academic community. We have all probably received unsolicited e-mails from publishers of journals, often with names very similar to highly respected journals, asking us to submit articles to that journal. Unfortunately once one undertakes some simple investigative work it soon becomes clear that these journals are not what they make out to be and that they are actually utilising an open access model of publishing to publish low quality articles. It is also apparent that many of these journals often do not adhere to the ethical guidelines published by COPE and/or ICMJE and that they are utilising a Gold Open Access author-pays model of open-access publishing for their own profit [14].

READERS

In the modern ethics in publication scenario the reader also has a role to play and should draw any suspected breach of ethics to the attention of the journal's editor by raising specific suspicions or comments, and if possible, supportive evidence. The journal editor should acknowledge this, and then instigate a suitable investigation into the claims and then follow up by advising the reader of the outcome of the investigation whether it is proved or not.

HOW SHOULD JOURNALS HANDLE PROBLEM PAPERS?

Once an investigation is completed there are a number of possible scenarios dependent on the

severity of the breach of ethics and whether the author is a repeat offender or not. If a breach of ethics is proven then the minimal, and expected, solution is withdrawal of the paper from publication and publication of a retraction notice. If the breach was severe and/or the author is a repeat offender then the publisher should consider banning the authors from publication in the journal for 3-5 years and informing the co-authors and editors of related journals of their action. For less serious cases, placing the author on a 'watch list' for careful examination of their submissions prior to requesting reviews may be applicable.

SUMMARY

On-line publication, open access or not, does not negate the need for ethics in publication. All those involved in the process must behave ethically be they Author, Reviewer, Editor, Publisher or Reader. In this way we can look forward to an era of open cooperation and dissemination of information to the benefit of all involved.

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In this issue: Focusing on Publication Ethics

The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

Open access publishing in the electronic age

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Key words:
open access, green, gold, platinum, business models, predatory publishing, laboratory medicine, eJIFCC

ABSTRACT

The principle of open-access (OA) publishing is more and more prevalent also on the field of laboratory medicine. Open-access journals (OAJs) are available online to the reader usually without financial, legal, or technical barriers. Some are subsidized, and some require payment on behalf of the author. OAJs are one of the two general methods for providing OA. The other one is self-archiving in a repository. The electronic journal of the IFCC (eJIFCC) is a platinum OAJ—i.e. there is no charge to read, or to submit to this journal. Traditionally, the author was required to transfer the copyright to the journal publisher. Publishers claimed this was necessary in order to protect author's rights. However, many authors found this unsatisfactory, and have used their influence to affect a gradual move towards a license to publish instead. Under such a system, the publisher has permission to edit, print, and distribute the article commercially, but the author(s) retain the other rights themselves. An OA mandate is a policy adopted by a research institution, research funder, or government which requires researchers to make their published, peer-reviewed journal articles and conference papers OA by self-archiving their peer-reviewed drafts in a repository ("green OA") or by publishing them in an OAJ ("gold OA"). Creative Commons (CC) is a nonprofit organization that enables the sharing and use of creativity and knowledge through free legal tools. The free, easy-to-use copyright licenses provide a simple, standardized way to give

the public permission to share and use creative work. CC licenses let you easily change your copyright terms from the default of "all rights reserved" to "some rights reserved." OA publishing also raises a number of new ethical problems (e.g. predatory publishers, fake papers). Laboratory scientists are encouraged to publish their scientific results OA (especially in eJIFCC). They should, however, be aware of their rights, institutional mandate, the procedures of publishing and post-printing, and the potential risks of OAP. Recent research shows that OA articles are wider seen, and are just starting to be better cited than equivalent papers published in traditional subscription journals.

1. INTRODUCTION

Open access (OA) is a model for publishing scholarly peer reviewed journals, made possible by the internet. The full text of OA journals and articles can be freely read, as the publishing is funded through means other than subscriptions. OA publishing actually presents a new opportunity to bring us closer to our authors and we are committed to providing more choices for them to publish and promote their research. Through OA, researchers and students from around the world gain increased access to knowledge, publications receive greater visibility and readership, and the potential impact of research is heightened. Increased access to, and sharing of knowledge leads to opportunities for equitable economic and social development, intercultural dialogue, and has the potential to spark innovation (Swan 2012, Boumil and Salem 2014, Pierce 2014). OA is the provision of free access to peer-reviewed, scholarly and research information to all. It requires that the rights holder grants worldwide irrevocable right of access to copy, use, distribute, transmit, and make derivative works in any format for any lawful activities with proper attribution to the original author. OA uses information and communication technology to increase and enhance the dissemination of scholarship. OA is about freedom, flexibility and fairness (Swan 2012).

2. THE GROWTH OF OA PUBLISHING

A study on the development of publishing of OA journals suggests that, measured both by the number of journals as well as by the increases in total article output, OA journal publishing has seen rapid growth particularly between the years 2000 and 2009. It was estimated that there were around 19,500 articles published OA in 2000, while the number has grown to 191,850 articles in 2009. The journal count for the year 2000 is estimated to have been 740, and 4769 for 2009; numbers which show considerable growth, albeit at a more moderate pace than the article-level growth. These findings support the notion that OA journals have increased both in numbers and in average annual output over time. The Registry of Open Access Repositories (ROAR: http://roar.eprints.org/) indexes the creation, location and growth of OA institutional repositories and their contents. As of May 2014, over 3,000 institutional and cross-institutional repositories have been registered in ROAR.

3. GREEN, GOLD, PLATINUM OA

3.1. OA repositories - the green route to OA

Authors who publish in scientific journals can share their research by posting a free draft copy of their article to a repository or website (Wiwanitkin and Qu 2014). This is referred to as green OA. This approach to OA involves additional effort from the author, as they will need to save the correct version of the article and post this to a repository, which will also need to add links and metadata to the hosted version of the article. No OA fee for authors because publication costs are paid for by library subscriptions.

Access is granted after an embargo period has expired rather than immediately, because libraries understandably will not subscribe if the content is available for free immediately. Policies should specify the maximum embargo length permitted and in science this should be 6 months at most: policies should require deposit at the time of publication with the full-text of the item remaining in the repository, but closed, until the end of the embargo period.

OA repositories house collections of scientific papers and other research outputs and make them available to all on the Web. They are all indexed by Google, Google Scholar and other search engines, so discovering what is in this distributed database is a simple matter of searching by keyword using one of these tools. Another successful subject-specific example is PubMed Central (PMC), the repository that houses the OA outputs of the National Institutes of Health amongst other things.

3.2. OA journals - the gold route to OA

OA journals are scholarly journals that are available online to the reader "without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Some are subsidized, and some require payment on behalf of the author. In the case of gold OA, the final version of record of an article is made free to read and re-use. Usually, there is a full reference linking, peer review process (though standards can vary). Tipically, an article publication charge is paid to cover publishing costs. Copyrights are usually regulated by Creative Commons license.

3.3. Platinum OA

Platinum OA is a model of scholarly publishing that does not charge author fees. The costs associated with scholarly publication are covered by the benevolence of others, such as through volunteer work, donations, subsidies, grants, etc. The term has been used for many years in numerous open-access publications, including books and blog entries, and on websites.

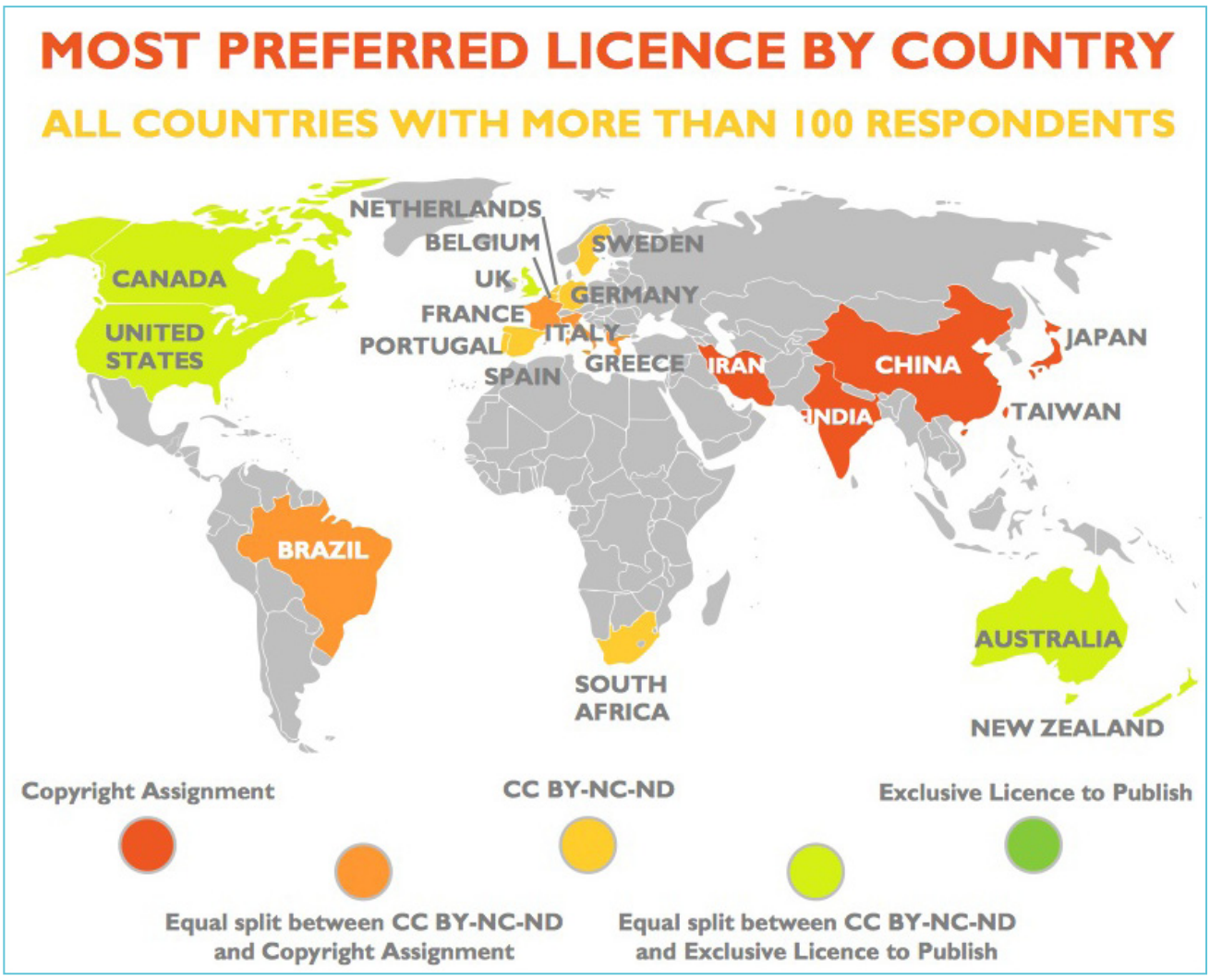
The Directory of OA Journals (DOAJ) is a website that lists OA journals and is maintained by Infrastructure Services for OA (IS4OA). The project defines OA journals as scientific and scholarly journals that meet high quality standards by exercising peer review or editorial quality control and "use a funding model that does not charge readers or their institutions for access. The Budapest OA Initiative's definition of OA is used to define required rights given to users, for the journal to be included in the DOAJ, as the rights to read, download, copy, distribute, print, search, or link to the full texts of these articles. As of 2014, the database contains 9794 journals, with an average of four to five journals being added each day. The aim of DOAJ is to "increase the visibility and ease of use of OA scientific and scholarly journals thereby promoting their increased usage and impact.

4. COPYRIGHT LICENCES WITH LIBRE OA - THE CREATIVE COMMONS

In order to reflect actual practice in providing two different degrees of OA, the distinction between gratis OA and libre OA was added. Gratis OA refers to free online access, and libre OA refers to free online access plus some additional re-use rights. The Budapest, Bethesda, and Berlin definitions had corresponded only to libre OA. The re-use rights of libre OA are often specified by various specific Creative Commons licenses (Creative Commons Attribution 4.0 International Public License, 2013) these almost all require attribution of authorship to the original authors. The Creative Commons copyright licenses and tools forge a balance inside the traditional "all rights reserved" setting that copyright law creates. Every license helps creators retain copyright while allowing others to copy,

distribute, and make some uses of their work—at least non-commercially. Every Creative Commons license also ensures licensors get the credit for their work they deserve. The CC BY license lets others distribute, remix, tweak, and build upon your work, even commercially, as long as they credit you for the original creation. This is the most accommodating of licenses offered. Recommended for maximum dissemination and use of licensed materials. The CC BY-SA license lets others remix, tweak, and build upon your work even for commercial purposes, as long as they credit you and license their new creations under the identical terms. This license is often compared to "copyleft" free and open source software licenses. All new works based on yours will carry the same license, so any derivatives will also allow commercial use. This is the license used by Wikipedia, and is recommended for materials that would benefit from incorporating content from Wikipedia and similarly licensed projects. The CC BY-NC license lets others remix, tweak, and build upon your work non-commercially, and although their new works must also

Figure 1 Most preferred Creative Commons license by country



A survey of the Taylor and Francis Group (2014). The survey has been distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

acknowledge you and be non-commercial, they don't have to license their derivative works on the same terms. The CC BY-NC-ND license is the most restrictive of our six main licenses, only allowing others to download your works and share them with others as long as they credit you, but they can't change them in any way or use them commercially. The CC BY-NC-SA license lets others remix, tweak, and build upon your work non-commercially, as long as they credit you and license their new creations under the identical terms. This license allows for redistribution, commercial and non-commercial, as long as it is passed along unchanged and in whole, with credit to you.

Most preferred Creative Commons licenses may vary from country-to-country, as revealed by a recent survey of the Taylor and Francis Group (2014).

5. BUSINESS MODELS

OA journals are published under a variety of business models (swan 2012). Costs need to be covered and there are various ways of doing this. Of course, the lower the cost base, the easier it is to develop a way of doing business that is sustainable. The main types of business model that allow a publisher to deliver OA content online are as follows (there are also examples of OA journal publishing that use combinations of these or variations on them).

5.1. Community publishing

This model is common for journals in small, niche areas of research. Journals are produced entirely within the academy and published online for free, and sometimes in print for a small subscription charge to cover the printing and distribution costs. The costs are kept at the lowest possible level by the use of volunteer labour for peer review, editing and production.

5.2. Advertising or sponsorship supported journals

The most likely area for attracting advertising is medicine and it is possible to find pharmaceutical companies that will sponsor a special issue of a journal or place regular advertising in a title. As well, the biggest names in academic journals outside medicine, Science and Nature, both attract large amounts of advertising from employers, conference organisers, other publishers.

5.3. Institutional subsidy

Institutions formally subsidise journal publishing wherever they are supporting a university press or a publishing operation by the library.

5.4. Hard copy sales

Many OA journals are published using this model, and thus have no need to levy an article-processing charge (APC) at the front end of the publishing process. All the contents are freely accessible online, and libraries around the world subscribe to the hard copy version.

5.5. Article-processing charges

It is commonly held that OA journals all levy a charge at the front end of the publishing process which has to be paid by authors, their institutions or research funders. This is not true. 53% of OA journals have no article-processing charges.

5.6. Institutional membership schemes

Some OA publishers have also introduced an institutional membership scheme.

5.7 Collaborative purchasing models

There is one example of such a model in the planning at the moment, the SCOAP3 (Sponsoring Consortium for OA Publishing in Particle Physics) venture in high energy physics.

6. PREDATORY PUBLISHING

In academic publishing, some publishers and journals have attempted to exploit the business model of open-access publishing by charging large fees to authors without providing the editorial and publishing services associated with more established and legitimate journals ("Beall's List", a regularly-updated report by Jeffrey Beall: http://scholarlyoa.com/publishers/), sets forth criteria for categorizing predatory publications and lists publishers and independent journals that meet those criteria. Complaints that are associated with predatory open-access publishing include:

- Accepting articles quickly with little or no peer review or quality control,[4] including hoax and nonsensical papers.
- Notifying academics of article fees only after papers are accepted.
- Aggressively campaigning for academics to submit articles or serve on editorial boards.
- Listing academics as members of editorial boards without their permission, and not allowing academics to resign from editorial boards.
- Appointing fake academics to editorial boards.
- Mimicking the name or web site style of more established journals.

7. OA PUBLISHING IN THE FIELD OF LABORATORY MEDICINE

OA publishing becomes increasingly popular also in the field of laboratory medicine. The number of OA journals is rapidly increasing. The Journal of The International Federation of Clinical Chemistry (eJIFCC) is a platinum OA journal with frequent updates on its home page. eJIFCC is an online journal, published four times a year, on the web site of the IFCC. The peer-reviewed original articles, posters, case studies and reviews, are focused on the needs of clinical laboratorians worldwide. In addition to the peer-reviewed content, there are also occasional editorials with pointers to quality resources on the Web. Also the journal publishes some IFCC news,letters, reviews of books, debates and educational material to assist the development of the field of clinical chemistry and laboratory medicine worldwide. The Editor welcomes suggestions of topics for review papers, and encourages submission of suitable original articles.

8. CONCLUSIONS

The Web offers new opportunities to build an optimal system for communicating science – a fully linked, fully interoperable, fully exploitable scientific research database available to all. Scientists are using these opportunities both to develop OA routes for the formal literature and for informal types of communication. For the growing body of OA information, preservation in the long-term is a key issue. Essential for the acceptance and use of the OA literature are new services that provide for the needs of scientists and research managers. OA is compatible with copyright, peer review, revenue (even profit), print, preservation, prestige, quality, career-advancement, indexing, and other features and supportive services associated with conventional scholarly literature. There are good, workable, definitions of OA and there is also a distinction made between two types of OA – gratis and libre – and this distinction also has policy implications. Two practical routes to OA (green and gold) have been formally endorsed by the research community.

Most authors, learned societies and editors agree that openness and public access to content are strongly to be desired. Although OA to research is a strong core value among many

academic and scientific communities, there are concerns, especially around economic and financial impacts, quality and peer review standards and licensing and reusability (variance between subjects). Through OA, researchers and students from around the world gain increased access to knowledge, publications receive greater visibility and readership, and the potential impact of research is heightened (Swan 2012).

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In this issue: Focusing on Publication Ethics

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How to write a scientific paper: practical guidelines

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ABSTRACT

Precise, accurate and clear writing is essential for communicating in health sciences, as publication is an important component in the university criteria for academic promotion and in obtaining funding to support research. In spite of this, the development of writing skills is a subject infrequently included in the curricula of faculties of medicine and allied health sciences. Therefore clinical investigators require tools to fill this gap. The present paper presents a brief historical background to medical publication and practical guidelines for writing scientific papers for acceptance in good journals.

INTRODUCTION

A scientific paper is the formal lasting record of a research process. It is meant to document research protocols, methods, results and conclusions derived from an initial working hypothesis. The first medical accounts date back to antiquity. Imhotep, Pharaoh of the 3rd Dynasty, could be considered the founder of ancient Egyptian medicine as he has been credited with being the original author of what is now known as the Edwin Smith Papyrus (Figure 1). The Papyrus, by giving some details on cures and anatomical observations, sets the basis of the *examination, diagnosis, treatment,* and *prognosis* of numerous diseases. Closer to the Common Era, in 460 BCE, Hippocrates wrote 70 books on medicine. In 1020, the Golden age of the Muslim Culture, *Ibn Sina*, known as Avicenna (Figure 2a), recorded the Canon of medicine that was to become the most used medical text in Europe and Middle East for almost half a millennium. This was followed in the beginning of the 12th Century by the extensive treatise of Maimonides (Figure 2b) (*Moses ben Maimon*) on Greek and Middle Eastern medicine. Of interest, by the end of the 11th Century Trotula di Ruggiero, a woman physician, wrote several influential books on women's ailment. A number of other hallmark treatises also became

Figure 1 **The Edwin Smith Papyrus (≈3000 BCE)**

This manuscript, written in 1600 BCE, is regarded as a copy of several earlier works (≈3000 BCE). It is part of a textbook on surgery the examination, diagnosis, treatment, and prognosis of numerous ailments. BCE: Before the Common Era.

Figure 2 Avicenna and Maimonides

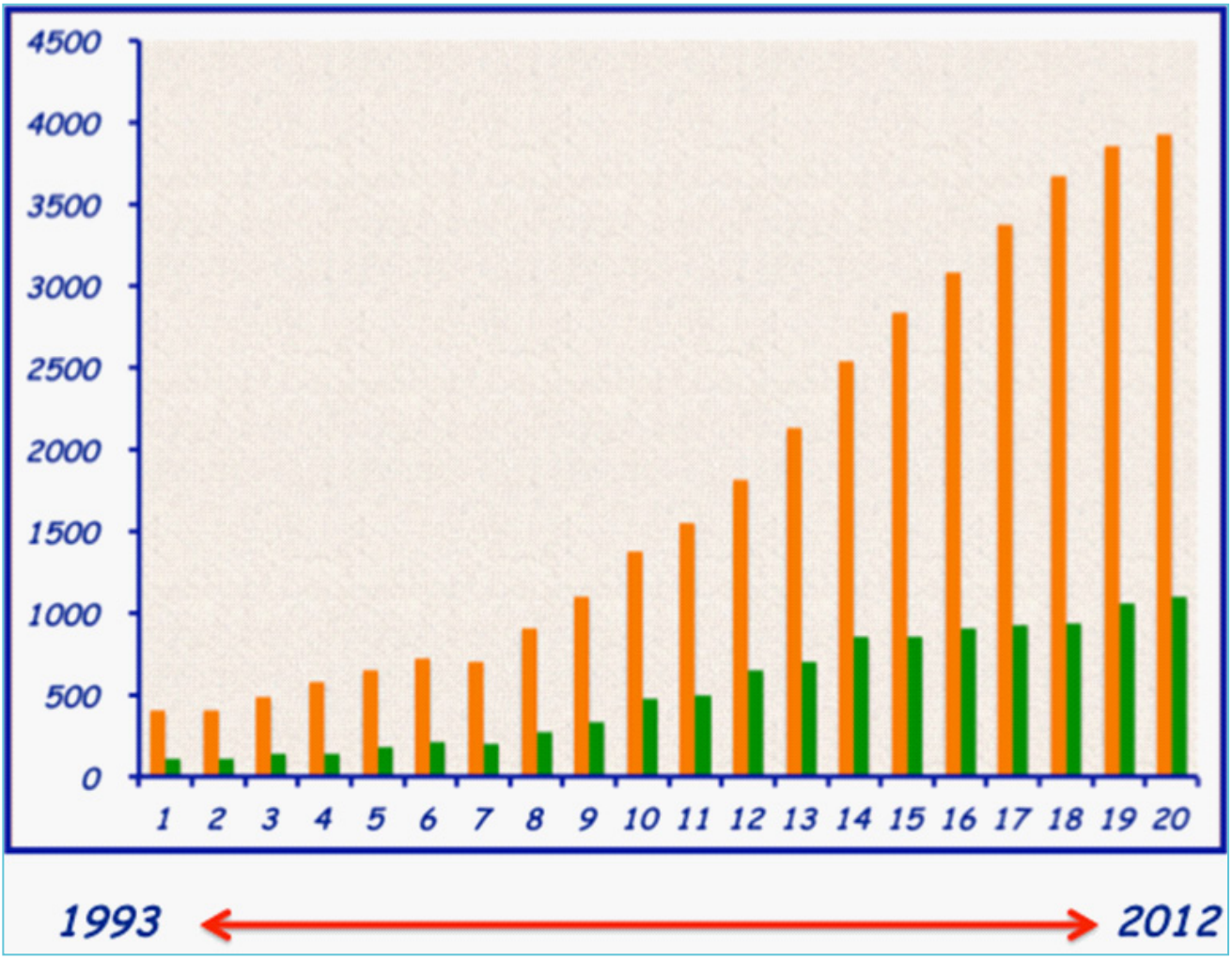
Figure 2a
Avicenna, 973 - 1037 C.E.

Figure 2b
Maimonides, 1135 - 1204 C.E.

more accessible, thanks to the introduction of the printing press that allowed standardization of the texts. One example is the De *Humani Corporis Fabrica* by Vesalius which contains hundreds of illustrations of human dissection. Thomas A Lang provides an excellent concise history of scientific publications [1]. These were the days when writing and publishing scientific or philosophical works were the privilege of the few and hence there was no or little competition and no recorded peer reviewing system. Times have however changed, and contemporary scientists have to compose with an increasingly harsh competition in attracting editors and publishers attention. As an example, the number of reports and reviews on obesity and diabetes has increased from 400 to close to 4000/year and 50 to 600/year respectively over a period of 20 years (Figure 3). The present article, essentially based on TA Lang's guide for writing a scientific paper [1], will summarize the steps involved in the process of writing a scientific report and in increasing the likelihood of its acceptance.

Reasons for publishing are varied. One may write to achieve a post-graduate degree, to obtain funding for pursuing research or for academic promotion. While all 3 reasons are perfectly legitimate, one must ask whether they are sufficient to be considered by editors, publishers and reviewers. Why then should the scientist write? The main reason is to provide to the scientific community data based on hypotheses that are innovative and thus to advance the understanding in a specific domain. One word of caution however, is that if a set of experiments has not been done or reported, it does not mean that it should be. It may simply reflect a lack of interest in it.

Figure 3 Annual publication load in the field of obesity and diabetes over 20 years.



Orange columns: *original research papers;* ***Green columns:*** *reviews*

DECIDING ON PUBLISHING AND TARGETING THE JOURNAL

In order to assist with the decision process, present your work orally first to colleagues in your field who may be more experienced in publishing. This step will help you in gauging whether your work is publishable and in shaping the paper.

Targeting the journal, in which you want to present your data, is also a critical step and should be done before starting to write. One hint is to look for journals that have published similar work to yours, and that aims readers most likely to be interested in your research. This will allow your article to be well read and cited. These journals are also those that you are most likely to read on a regular basis and to cite abundantly. The next step is to decide whether you submit your manuscript to a top-ranking impact factor journal or to a journal of lower prestige. Although it is tempting to test the waters, or to obtain reviewers comments, be realistic about the contribution your work provides and submit to a journal with an appropriate rank.

Do not forget that each rejection delays publication and that the basin of reviewers within your specialty is shallow. Thus repeated submission

to different journals could likely result in having your work submitted for review to the same reviewer.

DECIDING ON THE TYPE OF MANUSCRIPT

There are several types of scientific reports: observational, experimental, methodological, theoretical and review. Observational studies include 1) single-case report, 2) collective case reports on a series of patients having for example common signs and symptoms or being followed-up with similar protocols, 3) cross-sectional, 4) cohort studies, and 5) case-control studies. The latter 3 could be perceived as epidemiological studies as they may help establishing the prevalence of a condition, and identify a defined population with and without a particular condition (disease, injury, surgical complication). Experimental reports deal with research that tests a research hypothesis through an established protocol, and, in the case of health sciences, formulate plausible explanations for changes in biological systems. Methodological reports address for example advances in analytical technology, statistical methods and diagnostic approach. Theoretical reports suggest new working hypotheses and principles that have to be supported or disproved through experimental protocols. The review category can be sub-classified as narrative, systematic and meta-analytic. Narrative reviews are often broad overviews that could be biased as they are based on the personal experience of an expert relying on articles of his or her own choice. Systematic reviews and meta-analyses are based on reproducible procedures and on high quality data. Researchers systematically identify and analyze all data collected in articles that test the same working hypothesis, avoiding selection bias, and report the data in a systematic fashion. They are particularly helpful in asking important questions in the field of healthcare and are often the initial step for innovative research. Rules or guidelines in writing such report must be followed if a quality systematic review is to be published.

For clinical research trials and systematic reviews or meta-analyses, use the Consort Statement (**Con**solidated **S**tandards **O**f **R**eporting **T**rials) and the PRISMA Statement (**P**referred **R**eporting **I**tems for **S**ystematic reviews and **M**eta-**A**nalyses) respectively [2,3]. This assures the editors and the reviewers that essential elements of the trials and of the reviews were tackled. It also speeds the peer review process. There are several other Statements that apply to epidemiological studies [4], non-randomized clinical trials [5], diagnostic test development (6) and genetic association studies (7). The Consortium of Laboratory Medicine Journal Editors has also published guidelines for reporting industry-sponsored laboratory research (8).

INITIAL STEPS IN THE PROCESS OF WRITING A SCIENTIFIC DOCUMENT

Literature review is the initial and essential step before starting your study and writing the scientific report based on it. In this process use multiple databases, multiple keyword combinations. It will allow you to track the latest development in your field and thus avoid you to find out that someone else has performed the study before you, and hence decrease the originality of your study. Do not forget that high-ranking research journals publish results of enough importance and interest to merit their publication.

Determining the authorship and the order of authorship, an ethical issue, is the second essential step, and is unfortunately often neglected. This step may avoid later conflicts as, despite existing guidelines, it remains a sensitive issue owing to personal biases and the internal politics of institutions. The International Committee of Medical

Editors has adopted the following guidelines for the biomedical sciences (9).

"Authorship credit should be based only on: 1) Substantial contributions to the conception and design, or acquisition of data, or analysis and interpretation of data; 2) Drafting the article or revising it critically for important intellectual content; and 3) Final approval of the version to be published. Conditions 1, 2 and 3 must be all met. Acquisition of funding, the collections of data, or general supervision of the research group, by themselves, do not justify authorship." (9,10)

The order of authorship should reflect the individual contribution to the research and to the publication, from most to least (11). The first author usually carries out the lead for the project reported. However the last author is often mistakenly perceived as the senior author. This is perpetuated from the European tradition and is discouraged. As there are divergent conventions among journals, the order of authorship order may or may not reflect the individual contributions; with the exception that the first author should be the one most responsible for the work.

WRITING EFFECTIVELY

Effective writing requires that the text helps the readers 1) **understand** the content and the context, 2) **remember** what the salient points are, 3) **find** the information rapidly and, 4) **use or apply** the information given. These cardinal qualities should be adorned with the precise usage of the language, clarity of the text, inclusiveness of the information, and conciseness. Effective writing also means that you have to focus on the potential readers' needs. Readers in science are informed individuals who are not passive, and who will formulate their own opinion of your writing whether or not the meaning is clear. Therefore you need to know who your audience is. The following 4 questions should help you writing a reader-based text, meaning written to meet the information needs of readers [12].

What do you assume your readers already know? In other words, which terms and concepts can you use without explanation, and which do you have to define?

What do they want to know? Readers in science will read only if they think they will learn something of value.

What do they need to know? Your text must contain all the information necessary for the reader to understand it, even if you think this information id obvious to them.

What do they think they know that is not so? Correcting misconceptions can be an important function of communication, and persuading readers to change their minds can be a challenging task.

WRITING THE SCIENTIFIC PAPER

Babbs and Tacker's advice to write as much of the paper before performing the research project or experimental protocol may, at first sight, seem unexpected and counterintuitive [13], but in fact it is exactly what is being done when writing a research grant application. It will allow you to define the authorship alluded to before. The following section will briefly review the structure of the different sections of a manuscript and describe their purpose.

Reading the instructions to authors of the Journal you have decided to submit your manuscript is the first important step. They provide you with the specific requirements such as the way of listing the authors, type of abstract, word, figure or table limits and citation style. The

Mulford Library of University of Toledo website contains instructions to authors for over 3000 journals (http://mulford.meduoiho.edu/instr/).

The general organization of an article follows the IMRAD format (**I**ntroduction, **M**ethods, **R**esults, **a**nd **D**iscussion). These may however vary. For instance, in clinical research or epidemiology studies, the methods section will include details on the subjects included, and there will be a statement of the limitation of the study. Although conclusions may not always be part of the structure, we believe that it should, even in methodological reports.

Title page

The tile page provides essential information so that the editor, reviewers, and readers will identify the manuscript and the authors at a glance as well as enabling them to classify the field to which the article pertains.

The title page must contain the following:

- The tile of the article - it is an important part of the manuscript as it is the most often read and will induce the interested readers to pursue further. Therefore the title should be precise, accurate, specific and truthful;
- Each author's given name (it may be the full name or initials) and family name;
- Each author's affiliation;
- Some journals ask for highest academic degree;
- A running title that is usually limited to a number of characters. It must relate to the full title;
- Key words that will serve for indexing;
- For clinical studies, the trial's registration number;
- The name of the corresponding author with full contact information.

Abstract

The abstract is also an important section of your manuscript. Importantly, the abstract is the part of the article that your peers will see when consulting publication databases such as PubMed. It is the advertisement to your work and will strongly influence the editor deciding whether it will be submitted to reviewers or not. It will also help the readers decide to read the full article. Hence it has to be comprehensible on its own. Writing an abstract is challenging. You have to carefully select the content and, while being concise, assure to deliver the essence of your manuscript.

Without going into details, there are 3 types of abstracts: descriptive, informative and structured. The descriptive abstract is particularly used for theoretical, methodological or review articles. It usually consists of a single paragraph of 150 words or less. The informative abstract, the most common one, contains specific information given in the article and, are organized with an introduction (background, objectives), methods, results and discussion with or without conclusion. They usually are 150 to 250 words in length. The structured abstract is in essence an informative abstract with sections labeled with headings. They may also be longer and are limited to 250 to 300 words. Recent technology also allows for graphical or even video abstracts. The latter are interesting in the context of cell biology as they enable the investigator to illustrate *ex vivo* experiment results (phagocytosis process for example).

Qualities of abstracts:

- Understood without reading the full paper. Should contain no abbreviations. If abbreviations are used, they must be defined. This however removes space for more important information;

- Contains information consistent with the full report. Conclusions in the abstract must match those given in the full report;
- Is attractive and contains information needed to decide whether to read the full report.

Introduction

The introduction has 3 main goals: to establish the need and importance of your research, to indicate how you have filled the knowledge gap in your field and to give your readers a hint of what they will learn when reading your paper. To fulfil these goals, a four-part introduction consisting of a background statement, a problem statement, an activity statement and a forecasting statement, is best suited. Poorly defined background information and problem setting are the 2 most common weaknesses encountered in introductions. They stem from the false perception that peer readers know what the issue is and why the study to solve it is necessary. Although not a strict rule, the introduction in clinical science journals should target only references needed to establish the rationale for the study and the research protocol. This differ from more basic science or cell biology journals, for which a longer and elaborate introduction may be justified because the research at hand consists of several approaches each requiring background and justification.

The 4-part introduction consists of:

- A background statement that provides the context and the approach of the research;
- A problem statement that describes the nature, scope and importance of the problem or the knowledge gap;
- An activity statement, that details the research question, sets the hypothesis and actions undertaken for the investigation;
- A forecasting statement telling the readers what they will find when reading your article [14].

Methods section

This section may be named “Materials and Methods”, “Experimental section” or “Patients and Methods” depending upon the type of journal. Its purpose to allow your readers to provide enough information on the methods used for your research and to judge on their adequacy. Although clinical and “basic” research protocols differ, the principles involved in describing the methods share similar features. Hence, the breadth of what is being studied and how the study can be performed is common to both. What differ are the specific settings. For example, when a study is conducted on humans, you must provide, up front, assurance that it has received the approval of you Institution Ethics Review Board (IRB) and that participants have provided full and informed consent. Similarly when the study involves animals, you must affirm that you have the agreement from your Institutional Animal Care and Use Committee (IACUC). These are too often forgotten, and Journals (most of them) abiding to the rules of the Committee on Publication Ethics (COPE) and World Association of Medical Editors (WAME) will require such statement. Although journals publishing research reports in more fundamental science may not require such assurance, they do however also follow to strict ethics rules related to scientific misconduct or fraud such as data fabrication, data falsification. For clinical research papers, you have to provide information on how the participants were selected, identify the possible sources of bias and confounding factors and how they were diminished.

In terms of the measurements, you have to clearly identify the materials used as well as the suppliers with their location. You should also be unambiguous when describing the analytical

method. If the method has already been published, give a brief account and refer to the original publication (not a review in which the method is mentioned without a description). If you have modified it, you have to provide a detailed account of the modifications and you have to validate its accuracy, precision and repeatability. Mention the units in which results are reported and, if necessary, include the conversion factors [mass units versus "système international" (S.I.)]. In clinical research, surrogate end-points are often used as biomarkers. Under those circumstances, you must show their validity or refer to a study that has already shown that are valid.

In cases of clinical trials, the Methods section should include the study design, the patient selection mode, interventions, type of outcomes.

Statistics are important in assuring the quality of the research project. Hence, you should consult a biostatistician at the time of devising the research protocol and not after having performed the experiments or the clinical trial.

The components of the section on statistics should include:

- The way the data will be reported (mean, median, centiles for continuous data);
- Details on participant assignments to the different groups (random allocation, consecutive entry);
- Statistical comparison tools (parametric or non parametric statistics, paired or unpaired t-tests for normally distributed data and so on);
- The statistical power calculation when determining the sample size to obtain valid and significant comparisons together with the α level;
- The statistical software package used in the analysis.

Results section

The main purpose of the results section is to report the data that were collected and their relationship. It should also provide information on the modifications that have taken place because of unforeseen events leading to a modification of the initial protocol (loss of participants, reagent substitution, loss of data).

- Report results as tables and figures whenever possible, avoid duplication in the text. The text should summarize the findings;
- Report the data with the appropriate descriptive statistics;
- Report any unanticipated events that could affect the results;
- Report a complete account of observations and explanations for missing data (patient lost).

Discussion

The discussion should set your research in context, reinforce its importance and show how your results have contributed to the further understanding of the problem posed. This should appear in the concluding remarks. The following organization could be helpful.

- Briefly summarize the main results of your study in one or two paragraphs, and how they support your working hypothesis;
- Provide an interpretation of your results and show how they logically fit in an overall scheme (biological or clinical);
- Describe how your results compare with those of other investigators, explain the differences observed;
- Discuss how your results may lead to a new hypothesis and further experimentation, or how they could enhance the diagnostic procedures.

- Provide the limitations of your study and steps taken to reduce them. This could be placed in the concluding remarks.

Acknowledgements

The acknowledgements are important as they identify and thank the contributors to the study, who do not meet the criteria as co-authors. They also include the recognition of the granting agency. In this case the grant award number and source is usually included.

Declaration of competing interests

Competing interests arise when the author has more than one role that may lead to a situation where there is a conflict of interest. This is observed when the investigator has a simultaneous industrial consulting and academic position. In that case the results may not be agreeable to the industrial sponsor, who may impose a veto on publication or strongly suggest modifications to the conclusions. The investigator must clear this issue before starting the contracted research. In addition, the investigator may own shares or stock in the company whose product forms the basis of the study. Such conflicts of interest must be declared so that they are apparent to the readers.

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Kallikrein-related peptidases in prostate cancer: from molecular function to clinical application

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ABSTRACT

Prostate cancer is a leading contributor to male cancer-related deaths worldwide. Kallikrein-related peptidases (KLKs) are serine proteases that exhibit deregulated expression in prostate cancer, with KLK3, or prostate specific antigen (PSA), being the widely-employed clinical biomarker for prostate cancer. Other KLKs, such as KLK2, show promise as prostate cancer biomarkers and, additionally, their altered expression has been utilised for the design of KLK-targeted therapies. There is also a large body of in vitro and in vivo evidence supporting their role in cancer-related processes. Here, we review the literature on studies to date investigating the potential of other KLKs, in addition to PSA, as biomarkers and in therapeutic options, as well as their current known functional roles in cancer progression. Increased knowledge of these KLK-mediated functions, including degradation of the extracellular matrix, local invasion, cancer cell proliferation, interactions with fibroblasts, angiogenesis, migration, bone metastasis and tumour growth in vivo, may help define new roles as prognostic biomarkers and novel therapeutic targets for this cancer.

PROSTATE CANCER-ASSOCIATED DEREGULATION OF KALLIKREIN-RELATED PEPTIDASES (KLKS) AND THEIR USE AS CLINICAL BIOMARKERS

Prostate cancer is a leading cause of male cancer-related deaths in most developed nations. Although prostate cancer progresses through similar molecular and phenotypic 'hallmarks' as other endocrine-related cancers [1], the biological mechanisms driving its development are poorly understood. Locally-confined prostate tumours can be successfully treated by radical prostatectomy, androgen ablation and/or radiotherapy, although often with debilitating side effects. Despite the five-year survival rate for patients with localised tumours nearing 100%, there is a high degree of post-operative recurrence and many cancers progress to more advanced disease and ultimately incurable bone metastases [2]. Improved diagnostic and prognostic markers, as well as therapies, are required for effective prostate cancer detection and elimination.

Prostate cancer is accompanied by the aberrant expression of members of the KLK family of serine proteases, most notably KLK3 or prostate-specific antigen (PSA), which is the 'gold-standard' clinical biomarker for prostate cancer detection. PSA expression is largely prostate-specific, and this protease functions to liquefy the seminal clot in the healthy prostate [3]. In prostate cancer, although local PSA levels decrease with disease progression [4], serum PSA levels are elevated following its leakage into the bloodstream, resulting from a disrupted prostate glandular architecture. Total circulating PSA (tPSA) is measured in the 'PSA test' and, although there is no threshold for serum tPSA that definitively indicates prostate cancer, values ≥3-4ng/mL are generally accepted in the clinic, with a positive predictive value of ~25% at 3ng/mL [5]. Patients with serum tPSA ≥3-4ng/mL are often referred for biopsy to diagnose the cancer, after accounting for patient ethnicity, family history of disease and results of a digital rectal examination. PSA testing has reduced mortality of men [6]; however, it countributes up to 42% of prostate cancer over-diagnosis, which often translates to over-treatment [7]. Even with a high incidence of over-diagnosis and earlier diagnosis, many prostate cancers progress to form metastases, primarily in bone [2]. Clearly, there is a requirement for improved diagnostic and prognostic indicators of prostate cancer establishment and progression.

Attempts to refine PSA testing primarily centre around discrimination of various PSA iso-forms in circulation, including complexed PSA (cPSA), that is PSA complexed to other circulating proteins [8], free PSA (fPSA), that is PSA not bound to other circulating proteins [9, 10], full-length or intact PSA (iPSA) [9, 11], internally cleaved or 'nicked' PSA (N-PSA) [11], iso-forms, such as [2-]ProPSA [10], and various differentially glycosylated PSA proteins [12]. For example, measuring the Prostate Health Index (PHI; [2-]ProPSA/fPSA × √tPSA) improved predictive accuracy in patients with familial prostate cancer history and in men aged 60 and below [10, 13]. Discriminating tPSA, fPSA and iPSA, as well as measuring circulating KLK2, advanced the predictive accuracy of PSA testing, in addition to improving discrimination of pathologically insignificant from aggressive disease [9, 14]. This 'four kallikrein panel' is under further examination in a clinical trial for its ability to predict biochemical recurrence [15]. Thus, despite down-regulation of *KLK3* transcription in prostate cancer, certain post-transcriptional and -translational alterations to this peptidase appear to be enriched in diseased tissue, and detecting various PSA iso-forms may improve the specificity of clinical PSA testing. Additionally, as certain PSA regulatory pathways may be activated only in select

disease stages, discriminating between PSA isoforms may hold important prognostic value.

A number of other KLKs hold promise for prostate cancer diagnosis or prognosis, including *KLK4-5, KLK10-11* and *KLK14-15*. These KLKs, along with *KLK1-3*, *KLK9* and *KLK13*, are expressed and translated in prostate tissue [16] and have been detected in biological fluids, including serum (KLK1-8, KLK10-15) [17, 18], seminal plasma (KLK1-5, KLK7 and KLK9-15) [16] and extra-prostatic fluid (KLK1-3, KLK11 and KLK13) [19, 20]. *KLK2, KLK4, KLK11* and *KLK14-15* expression is increased in malignant prostate tissue, versus benign or normal tissue, and has been correlated with clinical disease parameters. *KLK4* expression is associated with increased risk of prostate cancer and tumour stage [21], and *KLK14-15* expression positively correlates with pathological stage [22, 23]. *KLK11* expression is increased in prostate cancer and inversely correlates with tumour stage and grade [24], while *KLK5* expression is inversely correlated with prostatic malignancy and Gleason score [25]. Additionally, DNA methylation of *KLK10* positively correlates with pathological stage [26]. Larger studies are required to confirm these findings and to determine whether changes in mRNA concentrations in prostate tissue correlate with reproducibly detectable differences in secreted protein abundance in biological fluids.

Overall, prostatic KLKs demonstrate useful biomarkers for prostate cancer. There is a clear clinical potential for measurement of the abundance of KLK4-5, KLK10-11 and/or KLK14-15 in biological fluids to improve prostate cancer diagnosis and prognosis. While prostate-specific expression of *KLK2* and *KLK3* is greater than other prostatic *KLKs* [27], prostate cancer-specific biomarkers, such as the aforementioned prostate cancer-associated KLKs, may be measured adjunct to prostate organ-specific biomarkers, for sensitive and specific detection of prostate cancer. However, larger clinical cohorts need to be evaluated before clinical translation of the apparent utility of these promising biomarker candidates. Identification of novel variants of these KLKs may serve to further enhance their biomarker potential, as has been demonstrated for PSA.

THE CLINICAL UTILITY OF KLK-TARGETED PROSTATE CANCER THERAPIES

Beyond their application as prostate cancer biomarkers, the tissue-specific, and/or deregulated, expression of KLKs has been utilised for the design of targeted cancer therapies. A range of anti-prostate cancer pro-drugs have been developed, whereby cytotoxic compounds have been coupled to KLK2- or PSA-activatable sequences, as the prostate-restricted expression of these KLKs allows for cytotoxicity selective to the prostate. Among these is L-377202, a PSA-activatable doxyrubicin-conjugate, which reduced tumour growth in a mouse model of prostate cancer and has completed Phase I clinical trials [28, 29]. KLKs also hold efficacy as antigens for immunotherapy. PROSTVAC® is one among available PSA-based vaccines, which is currently in Phase III clinical trials. It consists of vaccinia- and fowlpox-based vectors encoding transgenes for PSA and immune co-stimulatory molecules, which is administered to patients to elicit a T-cell response targeting PSA-expressing cells. Phase II clinical trials demonstrated that PROSTVAC® improved the overall survival at 3 years in men with low symptomatic multiple castration-resistant prostate cancer; progression-free survival was not affected [30]. Thus, novel therapies targeting the prostate cancer-enriched expression and/or activity of certain KLKs hold promise as cancer therapies.

Of note, an engineered variant of alpha-1-antichymotrypsin (MDPK67b), modified to inhibit a number of proteases, including KLK2, KLK4-5 and KLK14, is undergoing human trials [31]. This

represents the first KLK inhibitor as a putative prostate cancer therapy evaluated in a human study. Pre-clinical evidence showed that this inhibitor reduced tumour growth, conferred by KLK2 over-expression, in a xenograft model of prostate cancer. This compound exhibited low toxicity in the animal host [31]. The anti-tumour efficacy of inhibitors targeting other KLKs, demonstrated experimentally, must be confirmed in future clinical studies. To our knowledge, there are no other clinical studies targeting the biological function of KLKs in prostate cancer progression, despite various *in vitro* and *in vivo* animal studies showing functional roles for KLKs in this disease.

Converse to KLK inhibition, KLK agonists targeting those KLKs, which possess anti-tumourigenic activity, have been proposed as a therapeutic strategy. PSA-binding peptides have been developed, which serve as functional agonists of the anti-angiogenic and hence anti-tumourigenic activity of PSA in prostate cancer [32]. Similar agonists may be developed for other anti-tumourigenic KLKs, as such functions are discovered.

While KLKs show deregulated expression in prostate cancer, there has been minimal translation from laboratory-based evidence of the cancer-associated functions of prostatic KLKs, to clinical therapeutics targeting these functions. To bridge this gap and inform the design of therapies targeting KLK-mediated proteolysis, a greater understanding of the mechanism of KLK action in prostate cancer progression is required. In addressing this issue, the remainder of this review combines biochemical evidence of KLK-mediated substrate proteolysis with data from *in vitro* and *in vivo* animal studies, where KLK expression has been found to affect cellular 'hallmarks' of cancer (see Figure 1). In doing so, we provide a theoretical mechanism for KLK action in prostate cancer, which may form the basis for studies validating KLK activity in prostate carcinogenesis, from deregulated KLK expression, through proteolysis of their substrate intermediates and the affected down-stream signalling pathways, to the resulting functional outcomes. Only with such an understanding will the utility of KLKs as therapeutic targets for this disease be realised.

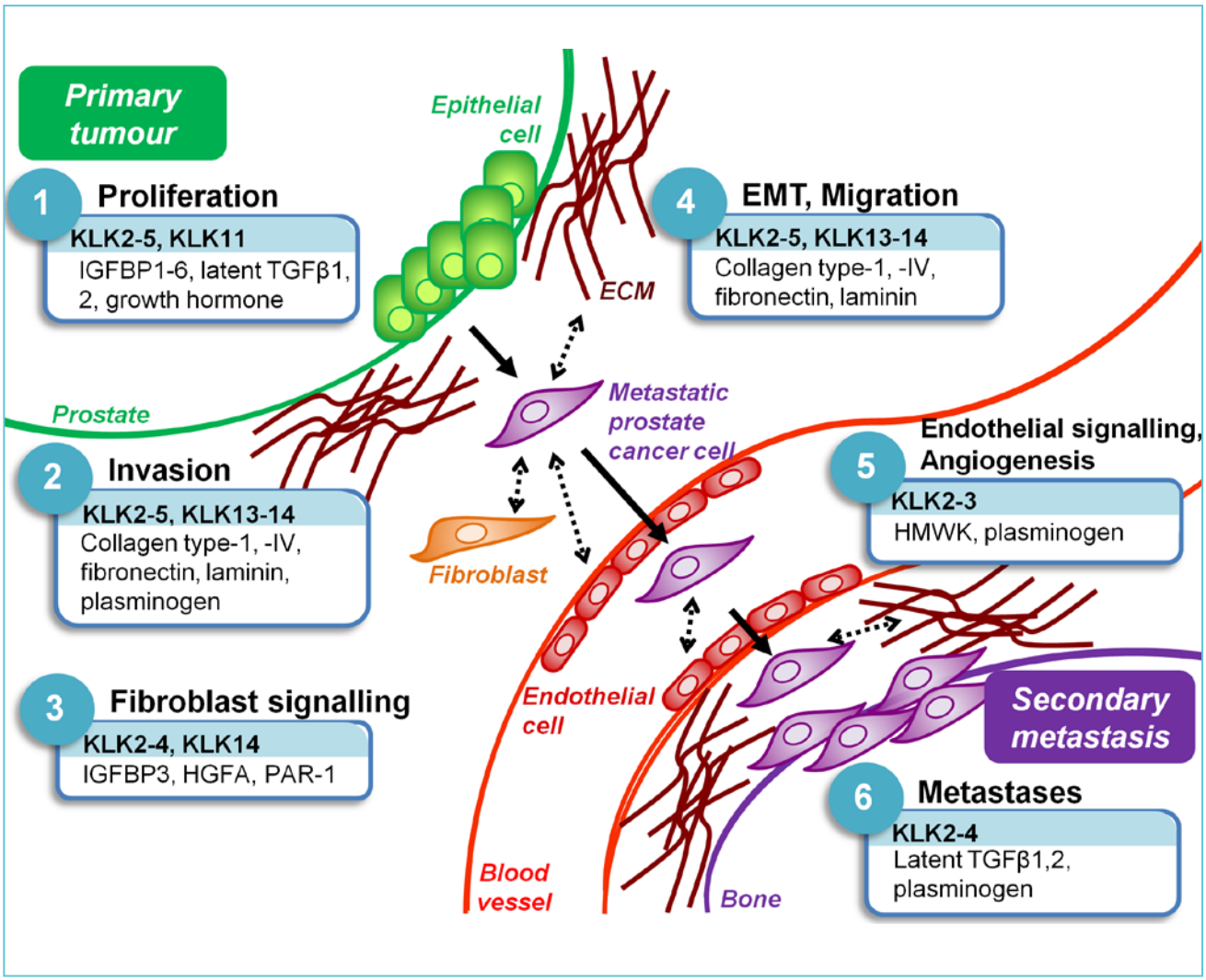
LABORATORY-BASED EVIDENCE FOR THE ROLE OF KLKS IN PROSTATE CANCER PROGRESSION

KLKs in extracellular matrix (ECM) degradation and local invasion

Primarily, the role of KLKs in degrading ECM proteins facilitates tumour expansion and invasion. The ECM plays a key role in tissue homeostasis, acting not only as a structural scaffold, but as a barrier to suppress malignant outgrowth, under healthy conditions. As with any tissue, ECM turnover is integral for healthy tissue maintenance, and a range of proteases, including KLKs, actively participate in this process. In prostatic malignancy, cancer proteases breakdown the basal lamina and facilitate physical clearance through the ECM to foster tumour outgrowth and entry into the vasculature [1].

Whether luminal or basal epithelial cells are the source of prostate cancer initiation is heavily debated as cancer cells often express mixed basal and luminal markers, the latter including KLK2 and PSA. KLK4, however, is expressed by both basal and luminal secretory epithelial cells and cleaves the basal lamina component, collagen type-IV, and the ECM components collagen type-I and fibronectin *in vitro* [33]. Thus, KLK4 is likely involved in the early breakdown of the basal lamina in prostate cancer. Luminal KLK2 and PSA may also function in this process, as cancerous outgrowth eventually brings cancer cells in contact with the basal lamina and the surrounding fibromuscular ECM. PSA cleaves laminin [34], while KLK2-4 also degrade

Figure 1 The role of KLK-related peptidase (KLK)-mediated proteolysis in prostate cancer progression.



KLKs implicated in 6 'hallmarks' of prostate cancer progression, by their in vitro substrates, are shown. KLK-expressing prostate cancer epithelial cells exhibit deregulated proliferation and invade through the surrounding basal lamina and extracellular matrix (ECM).

Cancer cells interact with stromal fibroblasts, as well as undergoing an epithelial-to-mesenchymal transition (EMT). These migratory prostate cancer cells interact with endothelial cells, lining blood vessels and gain entry to the vasculature.

Cancer cells must also activate blood vessel formation or angiogenesis, to supply oxygen and nutrients to the expanding primary tumour. Migrating prostate cancer cells extravasate at the compatible secondary site, bone, whereby they degrade the surrounding matrix and form osteoblastic metastases.

Arrows show the physical migration of a prostate cancer cell from the primary tumour to a secondary metastatic deposit. Dotted arrows represent KLK-mediated interactions between prostate cancer cells and cells or ECM in the surrounding microenvironment. Only those substrates referenced in the text are shown. IGFBP, insulin-like growth factor binding protein; TGF, transforming growth factor; HGFA, hepatocyte growth factor activator; PAR, protease-activated receptor; HMWK, high molecular weight kininogen.

fibronectin *in vitro*. Other prostatic KLKs also cleave fibronectin and laminin (KLK5 and KLK13-14), as well as collagens type-1 (KLK4-5 and KLK13-14) and type-IV (KLK4-5 and KLK14) [33, 35-37]. KLKs proteolytically process other KLKs and other protease classes *in vitro,* which may amplify KLK-induced ECM degradation, should this occur *in vivo* [38, 39].

Despite these *in vitro* observations, strikingly, bone metastatic prostate cancer, PC-3, cells made to over-express KLK2, PSA or KLK4 did not exhibit an altered invasive behaviour [40]. Additionally, and perhaps counter-intuitively, KLK4 over-expressing PC-3 cells showed increased attachment to collagens type-I and type-IV [41], although this could be a transient feature of migration and invasion. The proteolytic activity of KLKs secreted by these cells was not confirmed. KLK4 over-expressing prostate cancer cell lines generally display an enhanced migratory phenotype [40, 41] and PSA and KLK4 over-expressing PC-3 cells transition from an epithelial to a mesenchymal phenotype, characterised by loss of E-cadherin, gain of vimentin and acquisition of an elongated morphology, promoting increased migration *in vitro* [40].

KLKs and cancer cell proliferation

KLK-mediated ECM degradation can release matrix-tethered growth factors to facilitate cancer growth. Indeed, the direct or indirect activation of mitogenic proteins is key to sustained tumour growth [1]. Particularly, KLK2-5 and KLK11 degrade insulin-like growth factor binding protein 3 (IGFBP3) [35, 37, 42-44], KLK4-5 hydrolyse IGFBP4-6 [43, 45], and KLK5 and KLK14 process IGFBP2 [35, 37], with KLK5 also processing IGFBP1 [37]. Hydrolysis of IGFBPs can reduce the binding of these proteins to insulin-like growth factors (IGFs), thus increasing cell proliferation. Up-regulated levels of free, versus bound, IGF-1 positively correlates with prostate cancer occurrence [46]. Additionally, KLK2, KLK5 and KLK14 may activate latent transforming growth factor (TGF) β1, while PSA activates TGFβ2, which in turn act as tumour suppressors or promoters, depending on the tumour stage [47, 48].

KLKs degrade hormones and hormonal regulators, at least in a biochemical setting. For example, KLK4-5 and KLK13-14 cleave human growth hormone (GH). GH proteolysis from a 22kDa single-chain form to a disulphide-linked 2-chain form may impede cell proliferation and angiogenesis [49].

KLKs at the tumour-stroma interface: fibroblasts

The prostate epithelial cell microenvironment is a complex, dynamic milieu that plays an integral role in prostate cancer establishment and maintenance. The prostate stromal niche consists of a number of resident or recruited cell populations, including endothelial cells, pericytes, adipocytes, preadipocytes, fibroblasts, nerve cells, myofibroblasts, smooth muscle and immune cell populations. Stromal cells, particularly myofibroblasts, interact with healthy or transformed prostate epithelium, which passively or actively influences prostate cancer establishment and progression, through one or more of its 'hallmarks' [1]. KLKs expressed by the invasive tumour may interact with proteins from each of these cell classes to regulate the tumour microenvironment.

Myofibroblasts secrete a significant proportion of the ECM in the cancerous stromal niche, including many of the KLK-targeted ECM substrates outlined above. In addition to degrading fibroblast-derived ECM, KLK2 activates latent TGFβ1, one of the primary growth factors implicated in activation of the prostate cancer-adjacent fibroblasts, a process that renders fibroblasts permissive to, and accommodating of, prostate cancer growth [48, 50]. Prostatic stromal-derived fibroblasts respond to IGF signalling, and fibroblasts treated with PSA alone,

or combined with IGFBP3 and IGF-1, induced stromal fibroblast expansion, at levels additive of that induced by PSA and IGF-1 individually, thus abrogating the inhibitory effect of IGFBP3 on IGF-1-induced stromal cell growth. This activity was clearly proteolytic, because it could be abrogated by zinc inhibition [51]. KLK4-5 are able to activate the pro-form of hepatocyte growth factor activator, which subsequently activates stromal-derived hepatocyte growth factor to induce an invasive phenotype [52]. KLK2, KLK4 and KLK14 can activate protease-activated receptor-1, which is highly expressed on prostate fibroblasts, inducing mitogenic cellular response [53-55].

KLKs at the tumour-stroma interface: angiogenesis

Tumour-stroma interactions that are widely recognised as integral to cancer progression are those between tumour cells and endothelial cells, as well as neighbouring smooth muscle cells. KLK2 cleaves high molecular weight kininogen (HMWK) to release bradykinin, a factor that can induce smooth muscle cell contraction, facilitating vasodilation and cancer cell intravasation [56]. PSA releases a kinin-like molecule from seminal fluid, although this likely involves activation of a HMWK-activating intermediate, as recombinant PSA could not directly activate HMWK [57]. Conversely, PSA can cleave Lys-plasminogen to release bioactive angiostatin-like fragments, and these purified peptides inhibit human umbilical vein endothelial, HUVEC, cell tube formation [58]. PSA affects the expression of a number of HUVEC-derived genes, inversely regulating genes that are integral in tube formation [59]. Furthermore, PSA reduces the expression and production of the pro-angiogenic vascular endothelial growth factor, along with regulating other pro- and anti-angiogenic factors, in a metastatic derivative of bone metastasis-derived prostate cancer, PC-3M, cells *in vitro* and following subcutaneous implantation into mice [60].

Whether the role of PSA in angiogenesis depends on its proteolytic activity is heavily debated [61-63]. Inhibition of PSA activity with small molecule inhibitors abrogated its anti-angiogenic effects compared to active PSA alone[62]. It was postulated that PSA is the functional reason that prostate cancers grow slowly, relative to other cancers, given the dependence of expanding tumours on neo-angiogenesis [63]. This has led to the rationale of using PSA agonists as prostate cancer therapies [32].

KLKs in bone metastasis

The primary site of prostate cancer metastasis is the bone [2]. KLK2-4 are expressed in bone metastatic lesions; however, their expression is not necessary for cancer metastasis, but may be responsible for the predominating osteoblastic (bone-forming) versus osteolytic (bone-degrading) phenotype almost exclusive to prostate cancer [64]. KLK4 is perhaps the most interesting KLK with regard to bone metastasis, as ameloblast-expressed KLK4 degrades the dental enamel constituent, amelogenin, in maturing mouse molars, *in vivo* [65]. In humans, a mutation in the *KLK4* gene, predicted to encode a truncated KLK4 variant lacking a functional active site, is linked to amelogenesis imperfecta, a disease characterized by hypomaturation of the dental enamel [66]. Therefore, KLK4 is functionally active in mineralised dentine tissue, and by extension, is likely proteolytically active in the mineralised bone matrix of prostate cancer metastasis.

In accordance with the predilection of prostate cancer cells to metastasise to bone, KLK4 overexpressing PC-3 cells migrate preferentially to osteoblast-like, SaOS2, cell conditioned media

compared to vector controls, which is abrogated by the serine protease inhibitor, aprotinin [41]. Our group has identified a range of novel KLK4 substrates in a mineralised bone matrix model [67] and validation of the functional consequences of these interactions are underway (unpublished data).

Other KLKs may also function to promote bone metastases, through the aforementioned ability of KLK2 and KLK14 to cleave and activate latent TGFβ1, respectively, as TGFβ1 regulates osteoblast differentiation and bone formation. PSA may indirectly activate latent TGFβ1 by processing plasminogen into the TGFβ-activating protease, plasmin [58]. KLK2 and KLK4 activate pro-urokinase plasminogen activator (uPA), which activates plasminogen [39, 68]. KLK2 also activates PSA [39], and KLK4 activates both KLK2 and PSA [38, 68], thus providing a proteolytic cascade which amplifies TGFβ1 activation.

PSA is mitogenic for human and rodent osteoblast cell lines, which can be abrogated by the addition of a TGFβ1 and TGFβ2 neutralizing antibody [69]. PSA activates latent TGFβ2 produced by PC-3 cells [47], and incubation of PC-3 cell conditioned media with PSA induced proliferation of rat osteoblast-derived osteosarcoma, UMR106, cells, which was reversed by TGFβ neutralisation [70]. TGFβ1 has a well-recognised effect in bone formation, and PSA over-expressing rat prostate cancer, MatLyLu, cells, injected into mouse femur, displayed increased osteoblastic properties and decreased evidence of osteolysis, although tumour burden was the same as vehicle controls. At least one of the three subcutaneously inoculated PSA over-expressing clones showed evidence of PSA activity in murine sera compared to controls [71]. Active PSA directly injected into human bone, which had been subcutaneously implanted into mice, induced osteoblastic properties, such as increased bone volume, osteoid surface and osteoblast number, concomitant with a decreased osteoclast population [69]. Strikingly, the effect of PSA in osteoblast cell mitogenesis *in vitro* and osteogenesis *in vivo* was inhibited by serine protease inhibitors [69], suggesting that these outcomes were a result of PSA-mediated proteolysis.

KLKs and tumour growth in animal models

The roles of KLKs in tumour progression are confounded by their proteolytic activity not being reported in many cell culture or animal models, and, where reported, the discrepancy between levels of active KLK in these models as compared to patient tissue. PSA is believed to possess a high level of enzymatic activity around the prostate [72]. The efficacy of KLK2 and KLK4 activity surrounding the prostate is supported by known complex formations between active forms of these KLKs and serpins in seminal fluid, as serpins only inhibit active proteases [56, 73]. Androgen-sensitive prostate cancer, LNCaP, cells express KLK2-4 at higher levels than other commonly used prostate cancer cell lines; however, KLK2 and PSA in LNCaP secretions have low levels of activity [72]. Activity of endogenous KLK4 has not been confirmed in LNCaP or other cell lines. PSA knockdown reduced LNCaP cell proliferation *in vitro* and reduced tumour size by 10-fold relative to controls *in vivo* [74]. Despite these effects, levels of active PSA in xenografts are lower than those derived from patient tissue [72]. Knockdown of KLK4 in LNCaP cells similarly reduced xenograft tumour volume, although its proteolytic activity was not assessed [75].

To increase PSA activity in LNCaP cells to a level similar to that observed in human prostatic fluid, the wild-type PSA pro-region was substituted for a pro-region susceptible to activation by furin proteases, which are constitutively active in LNCaP cells. Subcutaneous transplantation of LNCaP cells over-expressing this furin-activating PSA construct into mice rendered circulating PSA, whereby 90% was active (bound to

serpins), compared to 12% being active in xenografts expressing wild-type PSA [74]. These tumours were increased in size compared to controls, validating *in vitro* findings that demonstrated increased cell proliferation [74]. To the contrary, when active PSA, at concentrations similar to that of patient tissues, was injected into tumours formed in mice upon subcutaneous injection of PC-3M cells, smaller xenografts were formed compared to saline-injected controls, concomitant with the down-regulation of platelet-derived growth factor β and uPA receptor [60]. PSA activity in these tissues post injection was not reported; hence, it is not known for how long, if at all, PSA retained activity.

CHALLENGES AND OPPORTUNITIES IN DEFINING KLK FUNCTION, TOWARDS TARGETING KLKS FOR CANCER THERAPY, AND THEIR UTILITY AS BIOMARKERS

KLKs show therapeutic promise as many prostatic KLKs drive cancer-related 'hallmarks', including epithelial-to-mesenchymal transition, migration, invasion, and local and distal stroma interaction, to facilitate tumour expansion and spread. PSA has served as the long-standing 'gold-standard' biomarker for prostate cancer, and there is evidence that other KLKs may add benefit as adjunct or stand-alone biomarkers for this disease. Particularly, KLK2 has shown the greatest clinical efficacy as a PSA-adjunct biomarker, in addition to discriminating between free, intact and total circulating PSA. This biomarker efficacy is emphasised by the effective application of KLK2- and PSA-activatable pro-drugs, the latter presently in clinical trials. Additionally, PSA appears to be an effective antigen for immune therapy. There exists only a single clinical trial targeting KLK function as a means of inhibiting prostate cancer progression, particularly using an engineered protein inhibitor of proteases, including KLK2, KLK4 and KLK14. Mapping the mechanism of KLK action in prostate cancer is the next step forward in the rationale design of targeted, novel therapies for this fatal disease.

To this end, laboratory-based studies of KLK function must address some key limiting issues. Firstly, it is imperative that the proteolytic activity of KLKs in cell culture and animal models be assessed in order to discriminate proteolytic from non-proteolytic functions. This is also important to determine if a lack of observed functional outcomes are due only to the inactivity of KLKs tested in these models. Furthermore, a current shortcoming, which presents also as a future opportunity for KLK research, is that functional studies to date nearly exclusively assess the action of a single KLK protease. However, multiple KLKs are simultaneously secreted by the cancerous prostate, and knowledge of the temporal expression and activity of each KLK, as well as redundancies in their proteolytic substrates and effector pathways, will be important for therapeutic design. Similarly, most functional studies have focused only on the epithelial cell component or the bone metastatic site. However, cross-talk of epithelial cell-derived KLKs with the local stroma, particularly activated fibroblasts, constitutes a myriad of interlinkages yet to be mapped that will likely greatly enhance knowledge of KLK function in prostate cancer. The next generation of cancer therapies will need to target both the tumour and activated stroma [76]; hence, it is crucial to outline those pathways in the latter compartment, as it is affected by deregulated KLK expression. Microenvironmental regulation of KLK activity is likely a key contributor to whether multifunctional KLKs act to promote or suppress tumour progression, at given disease stages. Thus, as much as possible, KLK function should be studied in humanised cell culture and animal models of prostate cancer to accelerate translation of key findings into the clinic.

Overall, understanding the functional consequences of deregulated KLK expression in prostate cancer will underpin the effective application and targeting of this protease family for prostate cancer treatment. This will also refine the utility of prostatic KLKs as prognostic biomarkers in these cancers if they are shown to be promising translational targets.

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Book Review
The new fundamentals: how less may mean more?

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REVIEWED BOOK

"Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics"
7th Edition, edited by Carl A. Burtis, PhD and David E. Bruns, MD.

RECENSION

Sometimes the name of a professional becomes a brand for some medical specialties and it is no surprise that readers call outstanding textbooks by the first authors name. This happenned with several authors like Kanski in ophtalmology and Colman in the field of haemostasis. Luckily laboratory medicine has its own brand name author: Norbert Tietz. Now, but there is the rub, what is actually laboratory medicine? This is extremely important when we cover this field in a textbook. According to recent intitiatives of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) our discipline should cover the vast repertoire of laboratory medicine. Even more so, in a recent survey it was figured out that aside from clinical chemistry a large percentage of the IFCC members also practice hematology, blood coagulation, immune diagnostics and many are also doing microbiology as well. Thus, it is likely that for specialists of our discipline and for our students the Tietz Fundamentals will not be the only source, however, it is probably the most widely experienced laboratory area and thus a particularly important source of learning.

The most recently completed volume of the new textbook of Clinical Chemistry and Molecular Diagnostics is proudly called as usual 'the Tietz Fundamentals'. Indeed, professor Tietz was extremely enthusiastic when, many decades

ago, he published the first edition of 'The Fundamentals of Clinical Chemistry'.

Aside from the many other disciplines that laboratorians practice today, it is evident, that 100% of all IFCC members experience clinical chemistry, so a new Fundamentals was surely required. Nevertheless we have to make distinctions between two series bearing the name of Norbert Tietz. The Fundmantals and the Textbook. There is a twofold difference in the volume of these series, but when one actually compares the content it is obvious that the Fundamentals is a really fine student friendly and professional condensation of the Textbook. It has also become evident in the past decade that even the fundamentals can not live without a session on molecular diagnostics, hence the seventh edition is comprising of this session in addition to the classical clinical chemistry part.

The structure of the book follows the well established chapters: (i) principles of laboratory medicine, (ii) analytical techniques and instrumentation, (iii) analytes (iv) pathophysiology (v) molecular diagnostics and (vi) reference information. All these were completed within the size of little over a 1000 pages. It is always difficult to decide what should be part of the 'fundamental knowledge' in clinical chemistry, this books tries to cover all state of the art parameters and when one does not find some lab tests among the analytes than the reader should look at the pathophysiology session where the significance of some assays–like cystatin C–are outlined with their pathophysiological contexts. Although many laboratories may not be able to do more esoteric tests, they should all do appropriate specimen collection, and avoid preanalytical errors as well as execute the clinical evaluation of the methods and do all clinical chemistry assays according to the basic principles of evidence based laboratory medicine. All these issues are covered in separate well-documented chapters at the beginning of the book dealing with the principles.

The outfit of the book is modest, each chapter is composed of the Objectives followed by the Key Words and Definitions and at the end of each chapter the reader finds a set of usually 10 review questions and the References. The printing techniques used red as a highlighting color and the easily comprehendable graphs are composed of three colors–black, white and red–and were created by using different brightness and contrast. This simple approach is very useful in clinical chemistry as it makes figures and graphs as simple as possible but no simpler like in case of chemical formulas that are indispensable requirements for a clinical chemistry book, the actual structure of the molecules (e.g. bile acids) and their converting enzymes can easily be identified by the different colors. Very helpful parts for laboratorians are the different algorithms e.g. in the investigation of disorders of water electrolyte and acid-base balance for the evaluation of volume status and osmolality.

However, it is somewhat unfortunate that some of the black and white photographs were adapted from previous editions and they might have needed some updating. Even more important for students are the fact that the techniques–very correctly listed–in this edition are utilized at a quite different extent in the recent practice of clinical chemistry. Most likely nobody uses rocket electrophoresis for serum albumin assay and also the double immundiffusion techniques has lost significance compared to the practice used in preceding years. Thus, one might consider some listed techniques as historical assays.

Naturally, the areas that improved the most in the past years has gained more pages in this new edition like the kidney diseases with description of the various forms of dialysis and

their effects on clinical chemistry values as well as the novel markers that had become routine for the bone and mineral disorders. An undoubtedly necessary chapter in recent clinical chemistry textbooks is the Pharmacogenomics session. This new Tietz Fundamentals dicusses this important issue as a prerequisite implicating personalized healthcare in our everyday practice of clinical chemistry. Only those drugs and prodrugs are discussed that are directly related to the field of clinical chemistry and enzyme phenotypes, while the many oncological drugs that also require molecular testing to predict their effectiveness are left for pathology textbooks. The final chapters are the Reference information for the clinical laboratory with conventinal and SI units and age-specific referenge ranges. As in all useful textbooks a Glossary terminates the book making it easy for students to understand all terminologies used.

All in all, it is always a good feeling to look into a new 'gold standard book' that was written by the numerous eminent contributors of todays laboratory medicine. We may only wish that our dicipline will be increasingly chosen by many young colleagues who will continue to benefit from such valuable sources.

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