Future of endometriosis research

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In women of reproductive age, health economic costs are estimated to be considerably higher for endometriosis than for conditions such as Crohn's disease, migraine and hypertension, and similar to the cost of diabetes. However, more awareness of endometriosis among patients and politicians is needed to create a better climate for research funding in the area of endometriosis in particular, and women's health in general. Recent collaboration between patients, physicians and politicians in the EU has shown that such efforts can be successful. Many arguments exist to organize the clinical care for women with advanced endometriosis in centers of excellence, but continuing education of primary-care physicians also remains a priority. New molecular techniques are resulting in new hormonal and nonhormonal targets for the noninvasive diagnosis and treatment of endometriosis. A future diagnostic serum assay might contain various elements from inflammatory serum markers to genetic/microarray/proteomics markers, owing to the multifactorial features of endometriosis.

Keywords: center of excellence, dioxin, endometriosis, noninvasive diagnosis, proteomics, treatment

future medicine part of fsg

Endometriosis is defined as the presence of endometrial-like tissue outside the uterus, which induces a chronic, inflammatory reaction. The condition is predominantly found in women of reproductive age, from all ethnic and social groups. It is associated with pelvic pain and infertility, which can impact on the physical, mental and social well-being of a woman and can have a profound effect on her life, including the ability to finish an education, maintain a career or to bear children. Endometriosis has been characterized as a polygenically inherited disease of complex multifactorial etiology [1]. Three main processes have been proposed as the origin of the disease. It is still difficult to decide which one of the retrograde menstruation, induction or inflammation theories is the best, but most of the evidence supports the retrograde menstruation theory [2]. Since the appearance of laparoscopy, a great boost has been given to surgical treatment, and it has become clear that the prevalence of endometriosis is more common than was previously thought. Awareness has increased among family doctors and patients regarding the medical and social implications of endometriosis [3,4]. Endometriosis has also gained some, but yet insufficient, attention in the public arena. The aim of this review is to highlight some areas of endometriosis where more research or action is needed, including basic research, noninvasive diagnosis, new medical treatment, and education of patients, physicians and politicians.

Cost, patient & government awareness, research funding & centers of excellence

Endometriosis is an expensive disease. In recent review papers, it has been reported that direct endometriosis-related costs are considerable, appear to be driven by hospitalizations, and increased by 61% between 1993 and 2002 in the USA, despite a decline in the endometriosis-related hospital length of stay during the same period [5–7]. Studies evaluating the cost of endometriosis in infertile patients and the indirect endometriosis-associated costs are largely lacking. Assuming a 10% prevalence rate of endometriosis among women of reproductive age, it has been estimated that the annual costs of endometriosis reached US$22 billion in the USA alone in 2002. In this age group, these costs are considerably higher than those related to Crohn's disease, migraine and hypertension, and are comparable with the cost of diabetes. Furthermore, endometriosis-related costs are increasing, in contrast to the decreasing direct costs associated with pelvic inflammatory disease. Owing to the added cost related to comorbid conditions such as interstitial cystitis, depression, migraine, irritable bowel syndrome, chronic fatigue syndrome, abdominal pain and infertility, women with endometriosis incurred total direct medical costs that were, on average, 63% higher than medical costs for the average women in a commercially insured group in the USA in 2004 [5–7].

10.2217/17455057.3.5.647 ISSN 1745-5057

The gap between the onset of endometriosis-associated symptoms and the diagnosis can be as long as 8.3 years [8]. This is a long delay despite the fact that the disease has a profound effect on the quality of life of women with endometriosis. Patient organizations are important to make endometriosis better known among women, to help and guide them, and to stimulate research. Indeed, patient information is crucial and may lead to a quick and precise information exchange, which in the long-term will reduce the diagnostic delay and mistreatment and will help the women going on with their normal life. Patient self-help groups can provide invaluable counseling, support and advice. The endometriosis.org website provides a comprehensive list of all the self-help groups in the world [101]. The 'Endometriosis Awareness Week' is an attempt to bring patients and doctors together and to promote public awareness of endometriosis.

There is also an increasing need for funding in endometriosis research. This is partly related to the fact that endometriosis is a disease of women, and most political decisions are still taken by men. Furthermore, endometriosis is not a 'sexy' disease, that is, it is not fashionable to promote endometriosis in the media, since it is related to taboo subjects such as menstruation and pain during intercourse, and also, endometriosis does not provide tragic episodes or miraculous cures since it is a benign disease. Another important reason why it has been difficult to obtain funding for endometriosis is because it is a benign (i.e., nonfatal) condition, even though it is a major health burden. Most funding towards studies into chronic diseases goes to the 'big killers' such as cancer and cardiovascular disease, and related conditions. More efforts are needed on a global scale to raise the awareness of the health consequences of this disease amongst politicians and the pharmaceutical industry, and to increase funding to better understand, diagnose and treat endometriosis.

In a recent paper, we have described how community action in Europe has addressed the need for more awareness and recognition of this social and economic effect of endometriosis [9]. Action, commenced at grass-root level, for increased awareness and investment in research has resulted in unprecedented recognition of endometriosis by the European Parliament, taken up by the Italian Senate in a 5-year action plan. This offers welcome assurance to the scientific community [9], and will hopefully lead to better funding for research in women's health at the EU level.

Recently, we have proposed to develop centers of excellence in endometriosis management and research [10]. Centers/networks of excellence are the only way forward to ensure that women with endometriosis receive consistent, evidence-based care, ensuring excellence, continuity of care, multidisciplinary, research, training and cost–effectiveness. Clinical excellence should be achieved by proper training, with adherence to evidence-based guidelines, quality management and continuous measurement of patient outcome as a central focus. To ensure continuity of care, the first step is to assign to each patient a 'central gynecologist' who must have continuously updated knowledge regarding all diagnostic and management options for endometriosis, and who must set priorities and realistic expectations together with the woman using a long-term multidisciplinary treatment plan. Scientific research within and scientific collaboration between centers/networks of excellence will create the critical mass of patients and tissue samples that is needed to make progress. Centers/networks of excellence should be accredited as training centers by professional bodies. They should aim at improving the cost–effectiveness of the management of endometriosis by a reduction in the time to diagnosis, a reduction in the time before individualized specialist care is invoked, a reduction of expensive 'hit-and-miss' treatments, and a reduction in expensive fertility treatments if the disease is under control before fertility is impaired [10]. However, it is also very important that generalists and gynecologists who perform primary care must understand endometriosis, which requires continuous education for primary-care physicians and gynecologists. If generalists and gynecologists who perform primary care are not able to diagnose endometriosis, such centers of excellence might not work well.

Finally, an international research body is much needed to identify and support the areas of endometriosis research that warrant further investigation, including also the areas of clinical research that are not in the primary interest of the pharmaceutical industry. For instance, the value of laparoscopic surgery for endometriosis before or after intrauterine insemination or IVF has been a matter of debate, mainly because large-scale, multicenter, randomized studies have not been performed. Recently, an international body, the World Endometriosis Research Foundation (WERF), has been created to address these issues. The WERF is based on a collaboration

between the World Endometriosis Society and the existing Special Interest Groups for Endometriosis at the European Society of Human Reproduction and Embryology and the American Society of Reproductive Medicine, which have a solid and transparent business and financial structure, and are devoted to the scientific and clinical progress of endometriosis by supporting postgraduate education and research.

Noninvasive diagnosis of endometriosis

Vaginal ultrasound is a useful method for diagnosis of ovarian endometriotic cysts and deeply infiltrative endometriotic noduli, but it is unable to reveal peritoneal endometriosis or endometriosis-associated adhesions [11,12]. The only reliable diagnostic method for endometriosis so far is laparoscopy [13], which is, for diagnostic use, expensive and puts an extra burden on both the operation room and the patient. Laparoscopic excision of endometriosis is not always an ultimate cure for patients, as recurrence rates after the operation can be as high as 40–50% after approximately 2 years [12,14,15]. At present, we recommend that subfertile women with a regular cycle, no pain, no male-factor infertility, normal clinical examination and normal pelvic ultrasound get a laparoscopy combined with hysteroscopy and tubal patency evaluation as part of their subfertility investigation. These women may have undetectable, extensive peritoneal endometriosis, with or without adhesions, that could cause subfertility and, possibly, mild pain, and surgery may improve both symptoms [11].

A noninvasive diagnostic test to detect minimal-to-mild endometriosis would be very important for a better identification of women who could really benefit from laparoscopic surgery. Many women with endometriosis are in their reproductive age and desire to become pregnant sooner or later. The early diagnosis of endometriosis by a noninvasive test could be followed by subsequent surgery, which could prevent the possible progression of endometriosis, resulting in more pain, infertility and in a declining quality of life [12]. For a clinical purpose, a diagnostic test with high sensitivity, ideally 100%, would be necessary even if the specificity were only 50%. Up to now, no such test has been developed [12]. Another vitally important use for a noninvasive diagnostic method will be in research, allowing the ability to carry out population-based studies elucidating etiology.

Several targets have been studied as potential diagnostic markers. A broad range of inflammatory cytokines show elevated concentration in the peripheral blood [16–18]. Unfortunately, so far, none of them has been developed into a fully fledged test because of their low diagnostic value. Tumor markers proven to be useful in the diagnosis of cancer (Ca19–9, CA-125 and CA15–3) have also been tested in women with endometriosis, but cannot be recommended currently [19,20]. The same can be said of proangiogenetic factors, such as VEGF, and C-reactive protein, a typical protein found in elevated quantity in inflammatory processes [21]. An additional problem in the quest for the ideal marker is that the serum concentration of molecules such as C-reactive protein and CA-125 varies during the menstrual cycle [21]. Therefore, it is crucial to carefully document the day and phase of the menstrual cycle in future studies assessing the value of serum tests in the diagnosis of endometriosis. Furthermore, the small differences that may exist between controls and cases require careful study design, with an appropriate sample number [22]. Occasionally, new cut-off levels need to be determined in order to improve diagnostic value [23,24]. Possibly, the serum analysis of a panel of markers, rather than one molecule, at a well-defined moment in the menstrual cycle could be beneficial [18], but this concept requires further study [20]. In addition, the value of semi-invasive diagnostic testing by analysis of peritoneal fluid [18], menstrual fluid or endometrial biopsies [25,26] requires more research [12].

Basic research

As mentioned in the introduction, endometriosis is a very complex, multifactorial disease. In order to understand its behavior and the differences that distinguish it from healthy tissue, scientists have to discover the fundamental mechanisms. This requires intensive basic research efforts and a meticulous integration of data from such distinct fields as inflammatory immunology, genetics, hormonal regulation and angiogenesis, among others. Since different factors play their role in the pathogenesis of the disease, possibly a certain number of features need to be combined to reach a critical threshold allowing the development of endometriosis [27].

Retrograde menstruation

What is the origin of endometriosis? Undoubtedly, this has always been the ultimate question in the investigation of the disease. Retrograde

menstruation is the most supported theory based on studies with women and baboons [28]. The quantity and quality of retrograde menstruation causing subclinical peritoneal fluid inflammation together with local peritoneal factors such as TNF-α, growth factors, interleukins and, most probably, other components that foster the adhesion of eutopic endometrium on the peritoneum could be a crucial factor in initiating the onset of the disease [15]. It is also suggested that the risk of recurrence of moderate-to-severe endometriosis is lower in women who have obstructed fallopian tubes as a result of endometriosis and are treated with IVF than in women who have open tubes and are treated with intrauterine insemination [29]. An investigation measuring 'objective menstrual blood loss' failed to find a difference between women with or without endometriosis. However, this measurement does not provide information regarding the volume of menstrual endometrium regurgitated into the peritoneal cavity [30].

Inflammation

The presence of inflammation is a well-known and commonly accepted observation [15,31]. Many cytokines and inflammation-related proteins have been found in elevated quantities in women with endometriosis compared with controls. Some of them have been discussed above in view of their potential for noninvasive diagnosis. Elevated numbers of scavenger cells were also reported in several papers [16,17]. One important question still remains unanswered: is inflammation a cause or a consequence of the disease? The early events of endometriosis that could provide information regarding these questions obviously can not be investigated in human patients. With the help of the newly improved animal models, for example the baboon model, it might be possible to shed light on these problems in the near future.

Attachment

The interaction between the endometrial tissue and the peritoneum could be a critical step in the settlement of the menstrual shedded tissue in the peritoneal cavity. It is not clear whether the intrusion of endometriotic tissue into the peritoneum is an active invasion of the endometrial cells or is related to active participation of the peritoneum itself. Studies show that normal endometrium has the ability to settle down onto the peritoneal surface regardless of the cycle phase. This attachment seems to be initiated by the stromal cell. Invasion-like phenotype starts to appear after 12 h of attachment in *in vitro* cell-culture experiments [32]. The capacity of endometrial cells to bind to the peritoneal surface varies widely among individuals [33], and also in women with endometriosis [34]. However, in a recent study, the attachment of human endometrial epithelial carcinoma cells (CRL-1671) to TNF-α-, IL-6- and IL-8-treated human mesothelial cells (CRL-9444) was inhibited by these inflammatory cytokines [35]. The concentration of these cytokines is elevated in the peritoneal fluid of women with endometriosis compared with controls [35,36], which makes the picture even more complex.

Steroids & endometriosis

Research has uncovered differences between normal and endometriotic tissue in the estrogen pathway. The expression of all enzymes in the estrogen biosynthesis process makes endometriotic stromal cells capable of autocrine estrogen stimulation [27]. Detailed analysis of regulatory proteins revealed a biased expression of gene-activator and -repressor regulators, which leads to the estrogen-production feature of endometriosis. Furthermore, other regulatory mechanisms, such as estrogen-neutralizing enzymes and progesterone receptors, are not present in endometriotic tissue [37]. Therefore, endometriotic cells are not only producing estrogen aberrantly, but they are unable to regulate and break the positive-feedback circles [27,37,38]. Many interactions between estrogen-production and inflammatory agents have been discovered [39]. By combining these new data, it is possible to draw a more subtle picture of the pathogenesis of endometriosis [27]. However, it is still unknown what causes the expression of enzymes supporting estrogen synthesis, and why proteins with a negative effect on estrogen production are absent in endometriotic lesions.

Genetics & molecular epidemiology

Endometriosis is also very likely a polygenetic disease. Heritability seems to be proven now [36]. Positional-cloning techniques provided direct proof of chromosomal linkage with region 26 on the long arm of chromosome 10, and other loci were also considered [40]. The use of microarray techniques in endometrial research has raised expectations regarding a new target for diagnostic markers and medical treatment and a better understanding of the pathogenesis of endometriosis [41]. Although the existing methods

have not yet solved the mystery of endometriosis altogether, microarray analysis of the endometrium has already generated large amounts of data regarding gene expression in different conditions. For example, Matsuzaki *et al.* analyzed deep endometriosis tissue using laser-capture microdissection to separate stromal and glandular cells in order to get a more subtle view of the system [42]. In another recent investigation, Wu *et al.* also used laser-capture microdissection on ectopic and eutopic endometrium from 12 women with endometriosis in the menstrual phase. They identified 904 genes/expressed sequence tags differences and 79 pathways, including focal adhesion, Wnt and MAPK signal pathway genes [43]. A more detailed generic overview sets forth some possible candidate genes for further investigations [44]. The most difficult part of the job still lies ahead: to identify gene-expression differences between tissues from women with and without endometriosis. A supportive high-throughput method for the microarray technology could be represented by the different matrix-assisted laser desorption/ionization and surface-enhanced laser desorption-ionization proteomics technologies, since the protein-expression pattern is also regulated at the translation level [45]. Much research is focused on genetic polymorphisms in susceptible genes and their regulating elements. These investigations are very important in discovering genetic differences at the basic genomic level, but research methodology is very important, as reviewed recently [46].

Environmental exposure & endometriosis risk

Environmental toxins, such as dioxins and polychlorinated biphenyls are some of the factors that have been suggested to play a significant role in the development of endometriosis [47]. Several research groups have studied the connection between these chemicals and endometriosis, but the results are very inconsistent [48]. There is only one paper, recently published, showing a significant positive correlation between deep endometriosis and dioxin-like polychlorinated biphenyls [49]. More studies using larger sample sizes are needed to confirm or refute this correlation, as proposed before [50].

Although the connection between environmental toxins and endometriosis is controversial, molecular mechanisms behind the dioxin effect have been investigated because dioxins have also been associated with other health problems, such as immunotoxicity [51]. Dioxin bound by its receptor, called aryl hydrocarbon receptor, is able to interact with estrogen receptor-α or -β. This connection leads to the activation of estrogen-responsive gene promoters [52]. Based on this observation, dioxin, as an estrogen agonist, can cause estrogen-dependent cell proliferation [52]. It is important to clarify the role of dioxins in the development of endometriosis, as environmental contaminants affect the entire population and government action would be required if there is proof that endometriosis is caused by exposure to environmental toxins.

Treatment of endometriosis

Considering the high recurrence rate currently associated with combined surgical and hormonal treatment, there remains considerable research interest in the medical treatment of endometriosis. Recently, new treatment possibilities have opened up, and this may be the most dramatically changing field within endometriosis research [53].

The discovery of local estrogen synthesis and related regulatory abnormalities has led to the application of aromatase inhibitors and GnRH analogues or oral contraceptives or their combinations.

Progesterone-related therapies such as selective progesterone-receptor modulators (SPRMs) may prove to be good alternative or supplementary agents for therapy [54]. Clinical trials in Phase II stage have recently been completed and their results are not yet available in the public domain [102].

Inflammation, as a key feature of endometriosis, provides a wide range of possible targets in therapy. Treatment with anti-TNF-α inhibitors has shown promising results in the baboon model [55,56]. Many other intervention possibilities can be considered based on the complexity of endometriosis. Some medical therapies aim at angiogenesis, another crucial step in the development and survival of endometriotic tissue [57]. Another approach is to aim at the invasion mechanism by blocking matrix metalloproteinases, the key enzymes in reconstructing the extracellular matrix [58]. All these examples clearly demonstrate the broad range of possibilities for therapies having emerged so far, although they are still in the phase of animal or *in vitro* testing. Many other drugs are in Phase II or Phase III trials, including new GnRH-receptor antagonists, oral contraceptives, estrogen-receptor-β agonists, and SPRMs, among others [102].

This research will hopefully lead to a wide diversity of treatments in the near future, allowing clinicians to choose an effective procedure for the patients. When more progress is achieved in understanding the pathophysiological behavior of endometriosis, even more therapeutic targets are likely to be discovered.

Future perspective

Basic research in endometriosis will be affected by a whole range of 'omics' technologies, which are currently being developed for upscaling from small-scale experimental studies to large-scale epidemiological studies: not only genomics, transcriptomics and proteomics, but also epigenetics (methylation of DNA) and, in particular, metabonomics (small molecules). These technologies could greatly improve endometriosis research, as it is a complex and multifactorial disease.

A noninvasive diagnosis for endometriosis is beneficial not only for the healthcare system, but for science as well, making it possible to design population-scale epidemiological studies to reveal the exact prevalence of the disease and possible ethnic differences among others.

Understanding the basic pathology of the disease will not only improve diagnosis and treatment, but will also allow for the identification of risk factors for endometriosis and the development of prevention strategies. Increased political awareness of endometriosis as a chronic benign gynecological disease, with a major impact on women's health, should lead to increased funding for research.

Financial disclosure

The authors have no relevant financial interests, including employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties related to this manuscript.

Executive summary

- Patient, public and political awareness of endometriosis is very important in order to improve research funding and clinical services for women with endometriosis.
- Endometriosis is a disease with a major economic impact.
- Noninvasive diagnostic tools are still missing. Several potential serum markers have been tested so far, but without considerable success.
- Basic research has several directions but no fundamental progress. Research aimed at revealing the problems of retrograde menstruation, attachment, immunological response and the broad analysis of RNA- and protein-expression differences between diseased and nondiseased tissues have a high potential for the development of new diagnostic and treatment options.

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Websites

101. Endometriosis support groups around the world
www.endometriosis.org/support.html
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