Implantation versus Infiltration: The Sampson versus the Endometriotic Disease Theory

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\textbf{Key Words}
Endometriosis \cdot Endometriotic disease theory \cdot Cellular mutation \cdot Implantation \cdot Metaplasia

\textbf{Abstract}
It has been assumed that endometriosis is a progressive disease, with growth and development of lesions being inevitable once the disease has started. The implantation and the metaplasia theories describe the mechanism of initiation of endometriotic lesions, but do not explain the different clinical manifestations of endometriosis. To explain the variable expression, growth and development of lesions into severe disease, a new endometriotic disease theory is proposed. This theory suggests that progression of endometriosis to endometriotic disease is considered similar to the onset and progression of a benign tumour. In this theory, the most important factor in the development of endometriotic disease is not the initial implantation/metaplasia, but cellular changes such as mutations. According to this theory, endometriotic disease develops from endometriotic cells that have ‘escaped’ the influence of protective and regulatory factors in the peritoneal fluid.

Adapted from the presentation given at the First Japan Conference on Endometriosis and extended from [1].

\textbf{Introduction}
Endometriosis has been defined by pathology as endometrial glands and stroma outside the uterus. These features have been demonstrated in various clinical lesions, ranging macroscopically from small nonpigmented vesicles, red vesicles, or polypoid lesions, to black puckered lesions in a sclerotic zone, the so-called typical lesions, and more severe lesions, such as deep lesions and cystic ovarian endometriosis. The pathophysiology and the natural history of the disease are poorly understood, whereas a comprehensive model, explaining the onset of the disease and all the different clinical manifestations is not available.

The actual theories and models are based upon the concept of a logical continuity between the different stages, as is the case for most diseases. It is assumed that growth and development is inevitable once the disease has started, and that endometriosis is a progressive disease, albeit progressing at different speeds in different women. Therefore research has focused mainly upon the pathophysiology of the onset of the disease, that is, the small and early lesions. These can be explained by implantation [2] or metaplasia [3, 4], and these theories are attractive since they are supported by abundant data [for review, see 5, 6].

It was known since the beginning of this century that menstrual endometrium contains viable cells [7–9]. After...
it became evident that retrograde menstruation is a frequent phenomenon [10], it was no surprise that peritoneal fluid contains viable endometrial cells [11, 12], which have the capacity to implant [13], to grow, and to infiltrate superficially [14]. The implantation theory was moreover supported by scar endometriosis [15], by the predominant localisation of the implants in the lower pelvis [for review, 16], and experimentally by transplanting endometrium in nude mice [17, 18], in primates by injection of endometrium or menstrual endometrium into the peritoneal cavity [19], or by obstructing menstrual outflow [20, 21], and in women by injecting endometrium subcutaneously [22]. The metaplasia theory [3, 4] or induction theory [23] was necessary, since the implantation theory cannot explain all localisations and manifestations of endometriosis [24–27], such as endometriosis in men or in women without retrograde menstruation [28–31]. For morphologic reasons, cystic ovarian endometriosis was suggested to start by metaplasia [32, 33]. The metaplasia theory is supported by the metaplastic capacity of the secondary müllerian system [34] and was proved experimentally, since menstrual debris can induce endometriosis [35]. A variant of the implantation theory is the spread through lymphatics and subsequent implantation [36].

Both theories, the implantation and the metaplasia theories, are mere variations of the mechanism of initiation of the endometriotic lesions. These theories do not explain the different clinical manifestations of endometriosis. To explain the variable expression of lesions and the variable growth and development into severe disease, several obvious possibilities have been explored. The peritoneal fluid, recognised as a specific microenvironment [for review, 6, 37, 38] could regulate growth and progression through its cytokines, growth factors, and others. The body defence mechanisms could be different, as evidenced by a decreased cellular immunity [39] or a lower natural killer (NK) cell activity (Oosterlynck D., PhD thesis: Natural Killer Cells and Endometriosis, 1993) [40–42]. The endometriotic cells were found to be different from those of the eutopic endometrium [43–48], and even the eutopic endometrium was found to be different in women with and without endometriosis [40, 49, 50]. These cellular differences, together with the hereditary [51–53] and genetic aspects [54–57] of endometriosis, which are well recognised, have been used to explain why endometriosis occurs in some women, that is, the onset of the disease, but not to explain the progression of the lesions, that is, to differentiate between implantation and progression.

Recently, evidence was gathered to consider subtle endometriotic lesions as a normal physiologic condition, occurring intermittently in all women [58–60], and to consider deep and cystic ovarian endometriosis a different entity [60]. To emphasise that the relationship between subtle endometriosis and pain or infertility is unclear, if not absent, whereas deep and cystic endometriosis is an obvious cause of pain and infertility [61], it was suggested to designate the latter as endometriotic disease. We therefore now want to focus upon the growth and progression of endometriosis into severe disease, as opposed to the onset, which can be explained by implantation or metaplasia.

The Endocrine Environment: Superficial versus Deep Lesions

It is obvious that superficial endometrial implants or lesions are influenced mainly by the local peritoneal fluid hormone concentrations. Peritoneal fluid is a specific microenvironment [62] with endocrine concentrations and with time courses that are different from those in the bloodstream [for review, 6, 38, 63]. Key factors identified are levels of ovarian sex steroid hormones, which are much higher than those in plasma [64], especially after ovulation [65]. This postovulatory increase does not occur in women with a luteinized unruptured follicle syndrome, known to be associated with endometriosis [for review, 38, 66]. Peritoneal fluid contains higher concentrations of macrophage-secretion products, especially in endometriosis with more and activated macrophages [for review, 6]. Many of these products, such as angiogenic factors, cytokines, and growth factors can easily be linked to growth or inhibition of superficial endometriosis, but the concept that peritoneal fluid could explain the development and growth into endometriotic disease has never been proven. Indeed, the overall effect seen in in vitro studies of peritoneal fluid upon the proliferation of purified endometrial stromal and epithelial cells was stimulatory, but no differences were found between women with and without endometriosis [67].

Possibly more important is the observation that the area of pelvic endometriosis is not directly, but inversely, proportional to the pelvic inflammatory macrophage response, since it suggests that the overall effect of peritoneal fluid upon growth and development of endometriosis may be inhibitory rather than stimulatory [68]. Considering the variety of secretory products, both concepts that peritoneal fluid may stimulate and inhibit growth of
superficial endometriosis, are not necessarily contradicto-
yry, since they might vary in some women and in different
conditions. The specific peritoneal fluid microenviron-
ment, as an ovarian exudate, is not a prerequisite for the
development of endometriosis, since endometriosis has
been described in women without ovarian activity, after
the administration of oestrogens [69].

To the best of our knowledge, no studies are available
that correlate clearly the type of endometriosis, that is,
only subtle implants, typical lesions, and well-defined
deep lesions or cystic ovarian endometriosis, and the
docrine peritoneal environment. Indeed, all studies use
the AFS classification, in which all stages are contami-
nated to a variable degree with deep endometriosis and
which does not differentiate between subtle and typical
lesions [for review, 66, 70].

Deep endometriotic lesions are regulated by blood-
stream factors instead of by peritoneal fluid factors. This
concept, that superficial endometriotic lesions are regu-
lated by peritoneal factors, whereas from a certain depth
wards these lesions are regulated by bloodstream fac-
tors, is self-evident. This concept is supported by the
observation that superficial lesions secrete CA125 and
PP14 mainly towards the peritoneal cavity, whereas deep
lesions secrete mainly towards the bloodstream [71]. From
the morphologic differences in deep lesions [61] and from
the biphasic frequency distribution of the depth of infiltr-
ation [72], the depth at which the influence changes from
peritoneal fluid to plasma has been estimated at 5–6 mm,
and this definition has been proposed as a definition of
deep endometriosis whether presenting as an infiltration, a
retraction or as adenomyosis externa [73].

In conclusion, one factor to understand the difference
in behaviour of superficial and deep endometriosis could
thus be to consider endometriotic disease as endometriot-
ic cells which have ‘escaped’ from the influence of perito-
eal fluid [for review, 74, 75].

**Immunologic, Cellular and Genetic Factors**

Women with endometriosis have a decreased cellular
immunity [39]. They specifically have a decreased NK
cell activity both in plasma and peritoneal fluid [40–42].
This decreased NK cell activity is not a consequence of a
decreased number, but of an inhibition of function. The
decrease in cellular immunity correlates with the severity
of the disease, by using the revised AFS classification,
that is, with the presence of cystic ovarian endometri-
osis. By two-way analysis of variance, the decrease in
NK cell activity correlates with both cystic and deep
endometriosis (D. Oosterlynck and P. Koninckx, unpubl.
results). Since women with minimal endometriosis do
not have a decreased cellular immunity, it is attractive to
postulate a causal relationship between decreased cellu-
lar immunity and severity of the disease. It remains
unclear however, whether this decrease in cellular immu-
nity is a cause or a consequence of the severity of the dis-
ease.

Endometriotic cells are different from endometrium
cells. These differences can, however, to a large extend be
explained by the different hormonal environment of these
cells. Whereas endometrium is influenced by bloodstream
factors, superficial pelvic endometriosis is regulated by
peritoneal fluid factors [71], and ovarian endometriosis
must be regulated by intra-ovarian concentrations, known
to be very different from plasma or peritoneal fluid. No
studies are available that investigate specifically differ-
ences between deep endometriotic cells and endometri-
um.

Endometrial cells from women with and without en-
dometriosis are different. Endometrium from women
with endometriosis is more resistant to NK cells [40], has
more P450 aromatase and interleukin (IL)-6 and IL-11
transcripts [49], demonstrates an increased expression of
heat shock protein 27 [76], and of angiogenesis [77].
These observations have, however, not been linked to the
severity of the disease, nor with the progression of the dis-
ease.

Endometriosis is more prevalent in first and second
degree relatives of women with endometriosis (for review
[57]). Also primate observations, and observations of the
onset of the disease in sisters [78], suggest a genetic
influence. Again no evidence is available to correlate this
with progression or severity of the disease.

**The Endometriotic Disease Theory**

This theory considers retrograde menstruation, viable
endometrial cells in peritoneal fluid, and occasional
implantation of some of these cells a normal physiologic
phenomenon. These nonimplanted and implanted cells are
normally removed by the defence mechanisms of the
body, such as macrophages. Attachment and implantation
is favoured when the mesothelial layer is damaged by
trauma, infection, or even by low-grade inflammation, for
example, irritation caused by carbon dioxide gas pneumo-
peritoneum, or by abundant retrograde menstruation. It
also seems logical to postulate that by mere statistical
mechanism, attachment and implantation must occur more frequently when more viable cells are present in peritoneal fluid. Although these cells can temporarily grow and develop depending upon the environment, their ultimate fate when left alone will be a spontaneous regression. This can be complete disappearance/removal or this can result in some fibrotic or scar tissue as the remnant of local inflammation, containing eventually some endometrial cells, shielded from the bloodstream, and some immunocompetent cells comparable to the bacteria in an abscess.

Endometriotic disease is caused by a cellular modification, for example, genetic mutation as observed in many benign tumours. This cellular accident will happen more frequently in genetically predisposed persons, and will be favoured by other factors such as total body irradiation, or chemical pollutants such as dioxins. It also seems logical that the probability that such a cellular accident occurs increases when more cells are present. The type of cellular modification, together with local factors such as the peritoneal fluid microenvironment or the intra-ovarian milieu, will determine whether they will develop into typical lesions, deep endometriosis or cystic ovarian endometriosis, and whether the morphologic characteristics will be chocolate cysts, endometrial glands and stroma, or adenomyosis externa.

Typical lesions thus are considered either as the remnant of a normal physiologic condition, or as a benign tumour with such a low invasiveness and growth potential that the lesions remain inactive over longer periods.

**Discussion**

The implantation/metaplasia theory fails to explain why deep and cystic endometriosis develop only in some women. According to these theories, we would expect a progressive increase in severity with age. These theories also fail to explain the variable expression of the disease. It is contradicted by the observation that endometriosis caused by outflow obstruction regresses spontaneously after removal of the obstruction [79]. Also, in the baboon model, the induced endometriosis decreased in most animals after a few months [19]. The implantation/metaplasia/progression theory seems to be contradicted by the clinical observation that minimal endometriosis is present in 60–80% of young women with infertility and pain, whereas ultimately only a minority will develop severe disease. Also the occurrence of very severe lesions in very young women is not compatible with the progression concept, since clinical observations (unpubl. data) suggest that these lesions left alone do not progress very rapidly.

Since minimal and mild disease can be considered a normal condition occurring intermittently in all women, and are not obviously linked to pelvic pain or infertility, it has been proposed to label them as ‘endometriosis’. This should mark clearly the difference with deep and cystic ovarian endometriosis, which are clearly a cause of pain and infertility. For clinical reasons, it thus was proposed to use ‘endometriotic disease’ to designate these forms of endometriosis that cause clinical symptoms [60].

The physiopathology of endometriotic disease is not known, and we only can speculate about factors that favour this development. Deep endometriosis can be considered as endometriosis that has escaped from the influence of peritoneal fluid, whereas cystic ovarian endometriosis could be the consequence of the local intra-ovarian environment [73]. Similarly, deep endometriotic lesions are also regulated by bloodstream factors instead of peritoneal-fluid factors. This concept, that superficial endometriotic lesions are regulated by peritoneal factors whereas from a certain depth onwards these lesions are regulated by bloodstream factors, is self-evident. It is moreover supported by the observation that superficial lesions secrete CA125 and PP14 mainly towards the peritoneal cavity whereas deep lesions secrete mainly towards the bloodstream. A decreased cellular immunity could be a cofactor in the development of endometriotic disease, but by absence of data this remains speculative, as the decreased cellular immunity could be caused by the endometriosis itself.

The endometriotic disease theory (EDT) is a new concept where progression of endometriotic disease is considered as the onset and progression of a benign tumour. In this theory the most important phenomenon in the development of endometriotic disease no longer is the implantation/metaplasia, but the cellular changes which cause the different behaviour. This genetic concept is supported by recent observations that cystic ovarian endometriosis is clonal in origin [80], that some endometriosis cells are invasive in vitro and have lost E adhesion receptors [81], a phenomenon which is well known in tumour biology.

The EDT is not contradicted by observations on endometriosis, but interprets them differently. ‘Severe endometriosis increases with age’ [72] no longer means progression, but is interpreted in analogy with most benign tumours that increase with age. ‘Endometriosis is a hereditary disease’ [57] no longer means that implantation is favoured, but that some cellular damages/injuries are
more likely to occur, similar to the BRCA1 association with breast cancer. ‘The endometrium is different in women with and without endometriosis’ is interpreted as a symptom of the different genetic constitution. ‘Endometriosis is an immunologic disease’ can mean that the decrease in cellular immunity and in NK cell activity can explain progression of disease, whether considered as implantation and progression or as mutation and progression. The EDT is supported by the observation that the decrease in NK cell activity in severe endometriosis does not change after surgery [82].

The same holds true for the idea that ‘endometriosis might be increased by dioxin’ [83], since most concepts on the mechanism of action suggest an immunologic effect. Considering, however, the direct genomic effect of dioxin, it would not be surprising if these substances would cause or be a cofactor in chromosomal damage. Angiogenic factors play a role in the development of endometriosis since in peritoneal fluid the bioassay angiogenic activity [84], transforming growth factor beta [85], and vascular endothelial growth factor [86, 87] concentrations are increased, whereas the vascularisation surrounding endometriotic implants suggest neoangiogenesis. Expression of angiogenic factors is well known in tumour biology, and can be explained easily by the EDT.

The expression of endometriosis is highly variable and the development of adhesions, cystic ovarian endometriosis or deep disease would reflect differences in the genomic alterations in the EDT. The same holds true for the morphologic appearance as glands and stroma, as chocolate cysts or as adenomyosis externa. In this view, typical lesions could reflect mutations with such a low grade of aggressivity that they do not progress, but sufficient to prevent destruction and removal by the immune system. The role of peritoneal fluid containing a variety of hormones and growth factor remains important for the local modulation of growth and development. Similarly, the ‘intra-ovarian’ milieu would be a cofactor to explain why large cystic ovarian endometriomas are found exclusively in the ovary.

The key event in the implantation theory is implantation, and in the EDT, a cellular mutation or other change. The clinical implications are that according to the implantation theory endometriosis is a recurrent disease and that women in whom the diagnosis of (minimal) endometriosis is made are considered at risk for developing severe endometriosis later in life. According to the EDT, minimal endometriosis, especially subtle or nonpigmented lesions, are no longer considered a pathologic condition and these women should not be considered as having an increased risk to develop severe endometriosis. Since the EDT considers endometriotic disease as a benign tumour, complete surgical excision would eradicate the disease with no risk of recurrence, whereas recurrence would imply incomplete excision or de novo occurrence of another lesion (at another location). The actual data showing recurrence rates after excision of severe and cystic ovarian endometriosis to be very low, are consistent with this view.

Acknowledgements

Mrs. Wolput is thanked for taking care of this manuscript. Mr. Benijts and Dr. Lempereur, Ipsen N.V. Belgium are thanked for their support. This work was partially supported by the NFWO Grant Nr. 1.5436.97N.

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The classic paper by Maher which reports on infertility associated with endometriosis does not make a distinction between subtle lesions and the more typical lesions. I think it would be very useful to have a separate analysis of women with subtle and typical lesions. When we investigated endometriosis in baboons we were able to show a link between the presence of the lutetising ruptured follicle (LUF) syndrome and typical lesions, and the association between the LUF syndrome and infertility is obvious. However no such association has been shown for subtle lesions and the LUF syndrome.

Prof. Koninckx: The definition was based upon pathological findings such as the number of mitoses. There was no direct relationship noted for active and inactive disease because this was based purely on the view of the pathologist at that moment. I think rather than classifying endometriosis as inactive or active disease based on activity in peritoneal fluid, we should move more towards using a tumor definition based on the degree of infiltration of the lesions. I believe that what we are seeing in peritoneal fluid is the consequence of this active endometriosis, which is not a disease, but rather a normal condition.

Prof. Hoshiai: Does this mean that you would prefer to use a different classification to that of the rAFS definitions, which, for example, bases its classification of active disease on the color of the peritoneal endometriosis?

Prof. Koninckx: Yes, I definitely think we need to move away from the rAFS classification system and classify deep lesions separately. The rAFS committee has discussed the need to distinguish between depth of lesions, but deep lesions remain absent from their present system.

Prof. Hoshiai: Several years ago we tried to distinguish between active and inactive endometriosis based on clinical features such as pain, or using a measurable index such as CA125 or cytokine levels in pelvic fluid. However we could not find any such factor to reliably...
differentiate between active and inactive disease. Do you have any comments on this difficult issue?

Prof. Koninckx: There are two biases in the literature. The first is the increased diagnosis of endometriosis based upon the presence of subtle lesions, which I think are a normal feature of many reproductive aged women. The second bias is the difficulty in diagnosing the presence of deep lesions. Of women with rAFS stage I and II disease, 30–40% will have deep lesions which are not diagnosed. Therefore, according to the rAFS classification, there is a very high incidence of deep lesions in what is called minimal and mild endometriosis. It is logical that by treating these patients, whose severity of disease is not recognized, they will benefit from treatment with a GnRHa. This leads to inaccuracies in interpretation of data investigating treatment of mild disease. The only way to improve on the diagnosis of these deep lesions is to systematically introduce a clinical examination during menstruation. In Leuven, I now refuse to operate on any women who have not had a clinical exam during menstruation.

Dr. Kanzaki: In your presentation you said that minimal endometriosis is a natural condition occurring intermittently in almost all women. Do you have any evidence supporting the natural disappearance of minimal endometriosis in women without pregnancy or hormone therapy?

Prof. Koninckx: There is no real hard evidence to show that minimal endometriosis disappears, the only data we have in this regard is on remodeling, which definitely occurs. What I have proposed is a hypothesis and a working model, different from the classical model, which looks at attachment and implantation as the important etiological events. In this new model, we look at genetics, immunology and infiltration of benign tumors.

Prof. Terakawa: According to your findings in the baboon and also in clinical findings, you mention that all women have mild endometriosis which can undergo remodeling and then disappear. Do you have any explanation why some lesions progress from these subtle lesions, moving on to become deep lesions?

Prof. Koninckx: I will address this issue in my second talk, but I would like to say that my working model for endometriosis is based on it acting exactly like a tumor. A tumor grows, until at some specific point it reaches a critical volume. Once the critical volume is reached the tumor outstrips the resources of the immune system and becomes aggressive. My suggestion for endometriosis is that peritoneal fluid generally inhibits the proliferation of endometriosis, but the moment the lesion escapes from the influence of this fluid, for instance when it is a deep lesion or cystic ovarian endometriosis, the behavior changes and it becomes aggressive.

Prof. Terakawa: You describe a type 1, type 2 and type 3 deep lesion and note that it is very difficult to find and clinically diagnose a type 2 lesion. Could you comment again on how best to diagnose this deep type 2 lesion.

Prof. Koninckx: In general, the type 1 lesions can not be felt in 20% of women, however they will not be missed on laparoscopy because they are such a large area of endometriosis. It is difficult to feel type 2 lesions because they are too high, above the uterosacral ligament. These lesions can often be seen on a bowel X-ray, which reveals distorted bowel. Laparoscopy in patients with type 2 lesions often reveal bowel which is adherent. If it is then incompletely freed at laparoscopy, it is possible to miss these lesions. They are only diagnosed during laparoscopy. It is for type 2 lesions that Americans perform a partial resection of the bowel and end-to-end anastomoses with the circular stapler. This tends not to be done in Europe because they consider this kind of aggressive bowel surgery overtreatment.

Prof. Taketani: I was interested to hear that deep infiltration can only be diagnosed during menstruation. Could you comment on why this is?

Prof. Koninckx: When a clinical examination is done during menstruation, those women with deep lesions find it extremely painful. You can feel these lesions during menstruation, a little bit during the first week and not at all during the last week of the cycle. Therefore, to find small deep lesions during laparoscopy, the laparoscopy has to be done immediately after, or even during menstruation. In late luteal phase, you can miss a series of deep lesions. It may be that the size of the deep infiltration fluctuates during the cycle. If you have a painful nodule during menstruation you always have a deep lesion or a cystic ovarian endometrioma which is attached to the uterosacral ligament.