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INVITED COMMENTARY

Fibrogenesis resulting from cyclic bleeding: the Holy Grail of the natural history of ectopic endometrium

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Innovation drought

Despite the exponential growth in the number of PubMed-indexed publications on endometriosis in the last 60 years (Guo, 2014), a simple reality check can be quite humbling and, perhaps more likely, disheartening. Innovation drought is conspicuous. So far not a single biomarker has been proven to be clinically useful for diagnosing endometriosis (Gupta *et al.*, 2016; Nisenblat *et al.*, 2016a, 2016b). Furthermore, a good disease-staging system that correlates with the severity of symptoms and/or has prognostic predictive capability is lacking.

The innovation drought is most glaring in the research and development of non-hormonal drugs: this is so painfully stagnant that the disappointment is audible (Vercellini *et al.*, 2011). Currently, the top-of-the-line drug for treating endometriosis is dienogest; a drug that was originally synthesized by Jenapharm in then East Germany in the 1979 well before the boom of molecular biology of endometriosis and certainly before the advent of genomics or proteomics. Dienogest is effective in suppressing endometriosis-associated pain; however, it only alleviates symptoms, it does not reduce the volume of the endometriotic nodules (Leonardo-Pinto *et al.*, 2017). While a new class of drug is now poised to get regulatory approval (i.e. gonadotropin-releasing hormone (GnRH) antagonists), it is far from innovative from the mechanism of action perspective even though it is structurally novel. GnRH antagonists still aims to modulate the hypothalamic–pituitary–gonadal (HPG) axis through a validated target, similarly to their agonist counterparts and, as such, may still share the same side-effects as GnRH agonists. Of course, GnRH antagonists can remove the 'flare-up' phenomenon, but their promise for more precise control of estrogen production could be undermined by the vast inter-individual variations. A search of ClinicalTrials.gov indicates that currently nine pharmaceutical companies are currently in the various stages of developing GnRH antagonists, making them the most coveted drug in reproductive medicine. Aside from GnRH antagonists, the research and development pipelines of all pharmaceutical companies seem to be trickling or are on the verge of drying up; several compounds in the Phase I stage appear to be congenitally ill-prepared to combat the fibroproliferative nature of endometriosis. No company is investing anything on drug research and development for adenomyosis, and so far all trials on non-hormonal drugs have apparently failed (Guo, 2014). Thus, it seems that in the next 5–8 years there will not be any truly revolutionary drug for patients with endometriosis or adenomyosis. This is a very serious problem.

What went wrong? Faced with such an abject failure, one could still pretend that all is well, continue business as usual, wait for divine revelation, and pray that some miracles will happen eventually. Conscientious and responsible investigators, however, may experience bouts of self-doubt, do some soul-searching, and then contemplate possible paradigm shifts. Realistically, questioning the very basic that we have espoused so dearly that might have shackled our minds and imagination would be an easy and rational first step.

Evolving definitions

Deceptively simple and straightforward, endometriosis has always been defined somewhat monolithically as 'the presence of endometrial glands and stroma outside of the normal location' (Beshay and Carr, 2012). However, this simple definition belies a kaleidoscopic variation in location, size, color, depth of invasion, presence or absence of adhesion, the proportion of epithelial/stromal cells, and even the presence or absence of these cell types, as in the well-documented phenomenon of 'stromal endometriosis' (i.e. lesions without glands) (Clement, 2007; Mai *et al.*, 1997). On top of these variations, there is also a wild variation in symptomology and severity among patients. Dense fibrotic tissues are often present in and/or surrounding the lesions (Bonte

et al., 2002; Clement, 2007; Cornillie et al., 1990; Guo et al., 2015; Itoga et al., 2003; Khare et al., 1996; Matsuzaki et al., 1999; Nisolle and Donnez, 1997; Stovall et al., 1992), especially in deep endometriosis (Bonte et al., 2002; Cornillie et al., 1990; Khare et al., 1996; Nisolle and Donnez, 1997). The fibrosis may result in subsequent adhesion, anatomic distortion and pelvic pain (Nisolle and Donnez, 1997). In this sense, the proposed redefinition of endometriosis by Dr Vigano and the famed Italian team in their Opinion article recently published in *Human Reproduction* is quite fitting and in fact long overdue (Vigano et al., 2018). With remarkable prescience, Vigano *et al.* rightly point out that a redefinition should reorient current research efforts towards more effective therapies, help to develop more adequate animal models for endometriosis and improve patient care (Vigano et al., 2018). Their proposal is all the more admirable at a time when one notable fad is to seek consensus on just about everything, because the proposal invites people to ponder, to think, to muse, and to quest for innovation, which is unlikely to come from any consensus.

In fact, the proposed redefinition by Vigano et al. (2018) that endometriosis is 'a fibrotic condition in which endometrial stroma and epithelium can be identified' could go a bit further based on evidence unearthed in the last few years.

As we now know, endometriotic lesions, or ectopic endometrium for that matter, are fundamentally wounds undergoing repeated tissue injury and repair (ReTIAR) (Guo et al., 2015; Zhang et al., 2016a, 2016b) due mostly to cyclic bleeding (Brosens, 1997). Consequent to this ReTIAR, the endometriotic lesions, stimulated by transforming growth factor (TGF)-β1 secreted from activated platelets and other immune cells or by some neuropeptides secreted from sensory nerve fibers, undergo epithelial–mesenchymal transition (EMT) and fibroblast-to-myofibroblast transdifferentiation (FMT), resulting in increased cellular contractility and collagen production, leading ultimately to fibrosis (Yan et al., 2017; Zhang et al., 2016a, 2016b). Prolonged exposure to activated platelets and/or immune cells also leads to increased expression of α-smooth muscle actin (α-SMA), as well as markers of differentiated smooth muscle cells (SMCs), by endometriotic stromal cells, which are likely to be responsible for the smooth muscle metaplasia (SMM) that is universally seen in endometriotic lesions (Itoga et al., 2003; Khare et al., 1996; Matsuzaki and Darcha, 2013; Mechsner et al., 2005). During disease progression endometriotic lesions also undergo epigenetic changes (Liu et al., 2017; Zhang, et al., 2017a), resulting in epigenetic aberrations as reported years ago (Guo, 2009).

Remarkably, similar processes apparently also occur in adenomyotic lesions owing, perhaps in no small amount, to the shared defining feature of cyclic bleeding as in endometriotic lesions (Liu et al., 2016; Shen et al., 2016). In other words, fibrogenesis is an integral and intrinsic part of the progression of ectopic endometrium; fibrosis is not just the secondary event triggered by an insult (Walton et al., 2017) – rather it is an inescapable destiny for wounds that undergo ReTIAR. While many details, such as the source of myofibroblasts, still await more research, these processes essentially depict the natural history of endometriotic lesions, which so far can only be guesstimated very roughly by the color of lesions (Brosens, 1994; Harirchian et al., 2012; Nisolle et al., 1993; Redwine, 1987), and, to a much lesser extent, the depth of invasion (Brosens, 1994).

In light of these developments, endometriosis may be better defined as 'a condition that started with the ectopic deposition of endometrial stroma and epithelium which undergo cyclic bleeding and thus repeated tissue injury and repair, resulting in gradual and progressive smooth muscle metaplasia and fibrogenesis'. One advantage of this definition is that it seems to cover all varieties of endometriosis and highlights the dynamic and progressive nature of endometriotic lesions. But more importantly, it essentially embodies the natural history of endometriotic lesions.

Implications

Arguably, knowing the natural history of endometriotic lesions holds the key to unlocking the enigma of the pathophysiology of endometriosis. Lesional fibrogenesis resulting from ReTIAR is essentially the Holy Grail of the natural history of ectopic endometrium.

The understanding of the natural history of ectopic endometrium should greatly empower endometriosis researchers. First, due to the shared commonality of cyclic bleeding, adenomyotic and endometriotic lesions can be investigated within the same framework (Liu et al., 2016; Shen et al., 2016). It has been long known that the two conditions often co-exist (Li et al., 2014; Leyendecker et al., 2015), and the two have been postulated to have the same origin (Leyendecker et al., 2002, 2009, 2015).

Second, it gives a global view of just how endometriotic lesions would develop and progress. This is important, since all too often we have seen a study that reports some molecular aberrations in endometriosis from painstakingly designed and meticulously executed experiments without knowing how the discovery fits in a global picture, much like a group of blind men groping and trying to figure out, in vain, what an elephant looks like. In addition, this global view could help to predict things that are otherwise difficult to find. Moreover, it may provide a framework to piece together seemingly unrelated findings, eventually weaving a complete tapestry of the pathophysiology of endometriosis.

Third, the natural history and the dynamic nature of several cell types involved in the process tell us that the cellular identity of lesions is simply not immutable. Rather, through interaction with other cells and mediators in their microenvironment, endometriotic cells may acquire a new morphology, new function, new phenotype and new identity, and collectively drive lesional fibrogenesis. The composition of cell types within a lesion may also change over time and this may explain why there are frequent conflicting reports in the literature because observations are often based on the use of mRNA or proteins extracted from a mixture of different cell types indiscriminately.

Fourth, through refocused research priorities, and perhaps also reallocation of resources, we can round up all perpetrators/suspects that are actively involved in promoting the lesional fibrogenesis, which can serve as potential drug targets. In the past, a great deal of efforts have been invested on hormonal and inflammation pathways without knowing what their roles are in lesional fibrogenesis. Since fibrosis is generally difficult to treat, let alone cure (Wynn, 2007), the fibrosis in endometriotic lesions is very likely to be chiefly responsible for the resistance to pharmacological treatment, especially in deep endometriosis (Koninckx et al., 2012). Conceivably, the low vascularization combined with the absence of steroid hormone receptors in the fibrotic tissues (Liu et al., 2017) are the major causes for the failure of the traditional hormonal drugs. As a corollary, any compound that does not have any anti-fibrotic capability may have little chance to succeed.

Sixth, by prudent choice of markers that represent various turning points in EMT, FMT, SMM and fibrogenesis it is possible to stage endometriosis by histology as has been shown in baboons (Zhang et al., 2016a, 2016b) and mice (Zhang, et al., 2017a, 2017b). A good histology-based staging system should be useful not only for prognostic purposes but also to provide a basis for precision medicine.

Seventh, by capitalizing on the advent of elastography, a novel imaging technique that can measure tissue stiffness, and on the intimate link between the extent of fibrosis and tissue stiffness, we can significantly improve our imaging diagnostic capability for endometriosis and adenomyosis, especially adenomyosis (Liu *et al.*, 2018) and deep endometriosis. It is also possible that, due to the correlation between lesional stiffness and hormonal receptor expression levels, results from elastographic imaging could be used to help choose the best treatment modality for deep endometriosis and/or adenomyosis (Liu *et al.*, 2018).

Eighth, seeing thorough the lens of this natural history of endometriosis, we can now understand why many clinical trials on endometriosis foundered. Despite enormous resources and toil invested, these trials are a parade of intrigue, surprise and disappointment, especially those trials on non-hormonal drugs (Guo and Evers, 2013; Guo, 2014). Surprisingly, there has been no open discussion on why and how these trials went bust, raising the prospect that many missteps and mistakes made in these failed trials would be repeated in future trials. With our current knowledge of the natural history of endometriotic lesions, it is easy to see that many, seemingly promising, preclinical studies that led to the launch of clinical trials (such as trials on inflixmab and ERB-041) used rodent or nonhuman primate models of endometriosis that did not fully recapitulate human endometriosis in general and fibrosis in particular. The only study that used a right baboon model, which is the closest to human condition, did get the right results (Barrier *et al.*, 2004). However, data interpretation in this study was in error: treatment with etanercept, a TNF neutralization agent, did reduce the amount of active lesions effectively, but it had no effect whatsoever on more advanced lesions. The latter observation is very likely replicated in the inflixmab trial (Koninckx *et al.*, 2008), leading to its demise.

Lastly, knowing the stage of progression can help us design far better studies to identify biomarkers for endometriosis, which so far has been a debacle (Nisenblat *et al.*, 2016a, 2016b). The information on the developmental stage of lesions should help boost the signal-to-noise ratio within the data, and perhaps also help to identify biomarkers for early-stage endometriosis.

Time for change

Dr Vigano and her associates are to be commended for their perceptive insight, timely proposal and for being the first to propose a change. In the face of innovation drought and all the debacles it is the right thing to do since 'old ways will not open new doors'. Starting with a new definition, especially with the understanding of the natural history of ectopic endometrium, there is a hope that things will turn around. It may be a much needed antidote to keep us sane and sober, since 'doing the same thing over and over again and expecting different results' are said to qualify for insanity.

Author's roles

The author wrote the paper on his own.

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Conflict of interest

The author has nothing to declare.

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