The Menstrual Endometrium: From Physiology to Future Treatments

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Abnormal uterine bleeding (AUB) is experienced by up to a third of women of reproductive age. It can cause anaemia and often results in decreased quality of life. A range of medical and surgical treatments are available but are associated with side effects and variable effectiveness. To improve the lives of those suffering from menstrual disorders, delineation of endometrial physiology is required. This allows an increased understanding of how this physiology may be disturbed, leading to uterine pathologies. In this way, more specific preventative and therapeutic strategies may be developed to personalise management of this common symptom. In this review, the impact of AUB globally is outlined, alongside the urgent clinical need for improved medical treatments. Current knowledge of endometrial physiology at menstruation is discussed, focusing on endocrine regulation of menstruation and local endometrial inflammation, tissue breakdown, hypoxia and endometrial repair. The contribution of the specialised endometrial vasculature and coagulation system during menstruation is highlighted. What is known regarding aberrations in endometrial physiology that result in AUB is discussed, with a focus on endometrial disorders (AUB-E) and adenomyosis (AUB-A). Gaps in existing knowledge and areas for future research are signposted throughout, with a focus on potential translational benefits for those experiencing abnormal uterine bleeding. Personalisation of treatment strategies for menstrual disorders is then examined, considering genetic, environmental and demographic characteristics of individuals to optimise their clinical management. Finally, an ideal model of future management of AUB is proposed. This would involve targeted diagnosis of specific endometrial aberrations in individuals, in the context of holistic medicine and with due consideration of personal circumstances and preferences.

Keywords: menstruation, endometrial, adenomyosis, abnormal uterine bleeding, inflammation, coagulation, vascular, hypoxia

INTRODUCTION

The symptom of abnormal uterine bleeding (AUB) is defined by the International Federation of Gynecology and Obstetrics (FIGO) as bleeding from the uterine corpus that is outside the normal parameters defined by AUB System 1 (Table 1) for duration, volume, frequency and/or regularity (1, 2). It encompasses heavy menstrual bleeding (HMB) and intermenstrual bleeding. AUB becomes chronic when symptoms are present for the majority of the preceding 6 months. AUB is a symptom and not a diagnosis. The FIGO AUB System 2 PALM-COEIN provides a system for diagnosing the underlying pathology resulting in the symptom of AUB.
Approximately one third of women of reproductive age experience AUB at some point in their reproductive lives (1, 2). PALM encompasses structural disorders such as polyps (AUB-P), adenomyosis (AUB-A), leiomyoma (AUB-L) and malignancies (AUB-M). COEIN refers to non-structural causes including coagulopathies (AUB-C), ovulatory dysfunction (AUB-O), endometrial disorders (AUB-E), iatrogenic causes (AUB-I) and not otherwise classified (AUB-N).

| Parameter                          | Normal                                      |
|-----------------------------------|---------------------------------------------|
| Frequency                         | 24–38 days                                  |
| Duration                          | Up to 8 days                                |
| Regularity                        | Regular variation (shortest to longest ≤ 9 days) |
| Flow volume                       | Normal                                      |
| Intermenstrual bleeding (bleeding between the cyclically regular onset of menses) | None                                        |
| Unscheduled bleeding on progestins ± oestrogen | Not applicable if not on hormonal medication or none |

It is thought that many women endure years of AUB that may result in anaemia, affect mental health and result in financial hardship (4). In the UK, over 800,000 women seek medical help for AUB annually and it is the fourth most common referral to UK gynaecological services (5). The impact amongst those who do not present for medical review is unquantifiable. It is thought that many women endure years of AUB that may result in anaemia, affect mental health and result in financial hardship (4).

There are a range of current medical treatments for AUB, but these are often limited by lack of effectiveness and intolerable side effects. Hormonal medications are the mainstay of medical management for AUB. These preparations act to override physiologic ovarian hormone production and do not specifically target the underlying cause of AUB in many cases. Hormonal medications have a range of contraindications and side effects and are particularly unsuitable for women who wish to conceive. Most importantly, women with AUB often find these treatments ineffective meaning that up to 60% of these women resort to fertility-ending surgical procedures with associated surgical risk (5). To improve medical strategies for AUB it is important to understand endometrial physiology and accurately diagnose the aberrations that result in AUB.

This review examines our current knowledge of menstrual physiology and the key processes involved. Aberrations which lead to AUB will be discussed, with a focus on AUB-E as an example of a non-structural cause and AUB-A as a structural diagnosis. Reference is also made to AUB-O, as most of the current treatments for AUB act at the ovarian level. However, detailed review of the processes and subsequent management of AUB –P/L/M/C/O/I/N is not included and we signpost readers to existing comprehensive reviews covering AUB-L (6), AUB-M (7) and AUB-C (8). Current management of AUB-A and AUB-E and its limitations will be reviewed, followed by discussion of recent advances and potential new therapeutic targets. Finally, a model of future management of AUB will be proposed with the aim of providing more effective, personalised treatments for those who are suffering with this debilitating symptom.

PHYSIOLOGY OF MENSTRUATION

The endometrium forms the inner lining of the uterus and requires an ability to change across the menstrual cycle to regenerate, decidualise, shed and to support implantation and pregnancy when necessary. In the absence of implantation, shedding of the luminal two-thirds of the endometrium occurs in a process known as menstruation. This occurs under the strict control of endocrine, immune, vascular and coagulation systems.

Physiology: Endocrine Regulation

The endometrium is a complex and dynamic multicellular tissue that responds to the ovarian hormones. Oestradiol is most abundant in the first half of the menstrual cycle, coincident with high rates of endometrial cell proliferation (9). Following ovulation, the endometrial secretory phase commences, where high levels of progesterone produced by the corpus luteum lead to altered endometrial morphology to prepare for implantation. If implantation does not occur, the corpus luteum regresses. Subsequent withdrawal of progesterone and oestradiol triggers a series of molecular and cellular events that resemble a classical inflammatory episode (pain, heat, redness and swelling) (10) and culminates with menstruation.

Physiology: Endometrial Breakdown and Inflammation

The progesterone withdrawal that occurs during the late secretory phase releases the transcription factor nuclear factor kappa B (NFκB) from its association with inhibitory proteins, such as IκB (11, 12). Once free, NFκB translocates to the nucleus, where it enhances the expression of inflammatory mediators (13, 14) including cytokines [tumour necrosis factor (TNF), interleukin-6 (IL6)] and chemokines [C-C motif chemokine ligand 2 (CCL2), interleukin-8 (CXCL8)] (13, 14). These mediators promote specific leukocyte trafficking and recruitment of myeloid cells (15). Activation of the NFκB pathway has been immunohistochemically detected in the endothelial, glandular and stromal compartments of the secretory endometrium (11). However, a thorough delineation of the particular cells types responsible for the initiation of the inflammatory cascade at menstruation is still lacking.

More recently, the effects of the inflammasome on the release of inflammatory cytokines during menses has been described, in vitro and ex vivo (16). The inflammasome is a multiprotein assembly that is classically associated with inflammatory signalling amplification (17). Both the NFκB and inflammasome systems may act simultaneously at menses to recruit immune cells to the endometrium. During menses, the most prevalent myeloid cells present in endometrial tissue are neutrophils and macrophages (18). Both myeloid cells activate and release matrix metalloproteinases (MMPs) in the endometrial milieu.
MMPs are widely accepted as being responsible for the shedding of the upper layers of the endometrium during menses (18), although the contribution of reactive oxygen species has also been suggested (19).

Once endometrial shedding has been accomplished, the inflammatory events which led to tissue destruction must be controlled in order to allow repair processes to begin. Several anti-inflammatory mediators emerge as candidates responsible for limiting the inflammatory response, including the glucocorticoid cortisol and lipid mediators.

Exposure of epithelial ovarian cells to the pro-inflammatory cytokine interleukin-1α (IL1A) has been found to increase hydroxysteroid 11-β dehydrogenase 1 mRNA (HSD11B1) (20). HSD11B1 catalyses the final step of cortisol synthesis, regulating the availability of this anti-inflammatory steroid. Similar regulation may be present in the menstrual endometrium, where the pro-inflammatory environment may activate anti-inflammatory pathways to resolve inflammation. Indeed, HSD11B1 mRNA was found to be increased in the endometrium at the time of menses (21), consistent with cortisol having a role in menstrual inflammatory resolution. A local increase in endometrial cortisol levels at menstruation may also result in a pro-repair environment. In vitro treatment of human endometrial stromal cells with cortisol was found to increase active transforming growth factor β (TGFβ) in cell culture supernatants (22), an ambivalent soluble mediator with context-dependent pro-inflammatory or restorative properties (23). Cortisol has also been shown to affect the macrophage secretome, with supernatant from cortisol-treated peripheral blood monocyte-derived macrophages resulting in altered endometrial endothelial cell expression of angiogenic genes C-X-C motif chemokine ligand 2 (CXCL2), CXCL8, connective tissue growth factor (CCN2), and vascular endothelial growth factor C (VEGFC) with a putative role in vascular repair (24). Cortisol has also been involved in the regulation of the platelet factor 4 (CXCL4/PF4) released by endometrial cells in vitro. This factor may be involved in endometrial repair by promoting the recruitment of reparative macrophages (25). Therefore, cortisol may limit the inflammatory response and create a pro-repair endometrial environment.

The presence of lipid mediators has also been associated with the resolution of inflammation. Specifically, lipoxins are lipid mediators with anti-inflammatory and pro-resolution properties that are present systemically (26). During menses, the lipoxin A4 receptor was increased in the endometrium at the mRNA level (27). Furthermore, in vitro studies showed that addition of lipoxin A4 to endometrial explants primed with an inflammatory stimulus mitigates the subsequent pro-inflammatory response (27). Hence, lipoxin A4 and other lipid mediators may play a role in limiting the inflammatory response within the menstrual endometrium and merit further study.

**Physiology: Limiting Blood Loss**

In addition to the resolution of menstrual inflammation, further mechanisms exist to limit menstrual blood loss. NFκB activation promotes the expression of prostaglandins and enzymes involved in their synthesis, such as cyclooxygenase-2 (COX2/PTGS2). While COX2 plays a role in endometrial breakdown (28, 29), prostaglandin F2α (PGF2α) (30), along with other vasoconstrictors like endothelin-1 (EDN1) (31), may curtail menstrual blood loss by constricting endometrial arterioles. Haemostatic mechanisms are also required to limit menstrual blood loss (8). During primary haemostasis, platelets adhere to the injured vascular endothelium and interact with the surrounding matrix, creating a platelet plug. The resulting platelet aggregation triggers activation of the coagulation system which, through complex interactions, converts soluble fibrinogen into an insoluble fibrin clot (32). In the endometrium, pre-clinical studies predict that platelet aggregation events are less crucial than vasoconstriction and fibrin clot formation (33). However, both the dysregulation of platelet aggregation and/or fibrin clot formation may have a negative impact upon menstrual blood loss (8).

**Physiology: Endometrial Repair and Regeneration**

After endometrial shedding, the denuded surface needs to be restored to minimise blood loss and recover its functionality for the next cycle. Endometrial hypoxia has been proposed as an important regulator of endometrial repair. Intensive vasoconstriction of the spiral arterioles during menstruation was directly observed in endometrial explants transplanted into the anterior chamber of the eye of rhesus monkeys by Markee in 1940 (34). More recently, markers of endometrial hypoxia have been detected in both in pre-clinical models (35–37) and the human endometrium (38, 39) during menses.

Although hypoxia does not appear to be required for endometrial breakdown (40), it may be important for triggering menstrual endometrial repair (37). Hypoxia inducible factor (HIF), is composed of an oxygen regulated alpha subunit (HIF1A) and a constitutively expressed beta subunit to form a transcription factor responsible for the cellular adaptive response to hypoxia (41). It is proposed that HIF1A is required for normal endometrial repair during menstruation, due to its exclusive presence in the perimenstrual phase, alongside evidence of delayed endometrial repair during menstruation with genetic or pharmacological reduction of HIF1A in mouse studies (37). HIF1A enhances the endometrial transcription of several genes involved in endometrial repair and blood vessel formation such as adrenomedullin, CCN2, CXCL8 and VEGF (42–44). Interestingly, some of these mediators can also be synergistically upregulated via prostaglandin action (44), which may represent dual regulation to ensure timely repair and cessation of menstrual bleeding.

**PATHOLOGY OF MENSTRUATION**

As described above, menstruation relies on meticulously coordinated endocrine, immune, vascular and haemostatic responses to limit blood loss and ensure optimal repair. Thus, repression or overactivation of the biochemical pathways involved in this process may result in pathological
manifestations. The role of (i) endocrine regulation, (ii) tissue breakdown and inflammation, (iii) vascular function and coagulation and (iv) endometrial repair in AUB is discussed below, with a focus on AUB-E and AUB-A.

AUB-E is a non-structural cause of AUB and is diagnosed when other causes of AUB have been excluded clinically. AUB-E represents an under-researched area where precise mechanisms resulting in this particular subtype of AUB remain undefined. AUB-A is an example of a structural cause of AUB. Adenomyosis develops a result of endometrium or endometrial-like tissue being present within the myometrial layer of the uterus. Adenomyosis may be asymptomatic or may cause symptoms such as dysmenorrhoea, subfertility or AUB (AUB-A) (45). Traditionally, adenomyosis has been diagnosed retrospectively following hysterectomy. There now is evidence to support ultrasound diagnosis of adenomyosis (46) but there remains a pressing clinical need to improve our understanding of the mechanisms causing AUB-A to improve diagnosis and management.

Pathology: Endocrine Regulation
Aberrant endocrine regulation of the endometrium is not known to be present in AUB-E or AUB-A but does occur due to ovulatory dysfunction (AUB-O). Menstrual disturbance in AUB-O occurs due to persistence of oestradiol signalling and lack of corpus luteum formation (47). The resulting lack of progesterone and subsequent progesterone withdrawal may result in heavy, infrequent and/or irregular menstrual bleeding. AUB-O occurs frequently at menarche and during peri-menopause or in those with polycystic ovary syndrome. Many current treatments for AUB act to override physiological ovarian hormone production and are often helpful in AUB-O. However, as endocrine dysregulation is rarely the primary cause of AUB-E and AUB-A, treatment failures occur and are discussed in further detail below.

Pathology: Endometrial Breakdown and Inflammation
Patients with AUB-E have been shown to have higher levels of TNF protein in their menstrual effluent when compared to those with normal menstrual blood loss (NMB) (48). TNF is a downstream inflammatory target in NFκB signalling, which can also act as an NFκB inducer (49). COX is another NFκB downstream inflammatory effector that is dysregulated in AUB. This biosynthetic enzyme possesses two isoforms (COX1/PTGS1 and COX2/PTGS2) and the mRNA of both were found to be increased in endometrium from those with AUB-E (50). This supports the hypothesis that excessive endometrial inflammation may be one mechanism causing AUB-E.

In the early stages of endometrial breakdown, local inflammation is tightly controlled. It may be hypothesised that those with AUB-E have disproportionate endometrial recruitment of neutrophils and macrophages that generate an inflammatory positive loop, where more mediators are released and further myeloid cells are recruited. In turn, these cells may over activate the secretion and release of MMPs leading to excessive or prolonged endometrial breakdown. Mechanistic preclinical studies focusing on the impact that upregulation of TNF, COX and other NFκB-induced inflammatory mediators have on endometrial leukocyte recruitment and MMPs activation are needed to confirm or refute this hypothesis.

An altered inflammatory response may also contribute to the symptom of AUB-A. In the presence of adenomyosis, NFκB binding activity is constitutively overactivated both in the eutopic endometrium (51) and adenomyotic lesions (51, 52). The increase in inflammatory cytokines released by leukocytes isolated from both the eutopic endometrium and adenomyotic lesions of women with adenomyosis compared to healthy controls (53) also suggests inflammatory dysregulation. Moreover, NFκB activity has been positively associated with the symptom of AUB-A (54). Interestingly, in those with AUB-A, COX2/PTGS2 mRNA levels both in the adenomyotic lesions and the eutopic endometrium are increased compared to adenomyotic patients with NMB. This COX2/PTGS2 increase correlated with higher expression of the pro-inflammatory mediators IL6 and CXCL8 (55), consistent with a pro-inflammatory endometrial environment increasing menstrual blood loss in those with adenomyosis.

As previously discussed, endometrial cortisol may play a role the resolution of the menstrual inflammatory response. Patients with AUB-E have been shown to have an increased endometrial expression of the cortisol-inactivating enzyme hydroxysteroid 11-β dehydrogenase 2 (HSD11B2) (56) as well as a decrease in the downstream cortisol target CXCL4/PF4 during menses (25). Hence, cortisol deficiency may play a key role in AUB-E. At present, there are no studies exploring a potential correlation between cortisol levels and AUB-A.

Pathology: Limiting Blood Loss
As described above, arteriole vasoconstriction is a key process to limit menstrual blood loss. Defective vasoconstriction may have its origin in alterations in vasoconstrictive mediators. In AUB-E, a decrease in the vasoconstrictor EDN1 at the protein level has been described, as well as an increase in the enzyme responsible for its inactivation, neutral endopeptidase (57). There is also evidence of higher endometrial levels of the vasodilating prostaglandin PGE2 in those with HMB compared to NMB (50). These effects in conjunction with decreased mRNA levels of the PGF2α receptor (PTGFR) (58), cause a reduction in the ratio PGE2/PGE2 which may result in a defective ability to limit menstrual blood loss (50, 58).

In patients with AUB-A, there are no reports of endometrial differences in EDN1 or prostaglandins. Studies are required to examine the eutopic endometrium in those with AUB-A and control groups of those with adenomyosis who do not experience AUB and those free from disease and symptoms. However, within adenomyotic lesions, the vasoconstrictor/vasodilator balance appears to be disrupted. Preliminary data from in vitro studies (59) show that cells from adenomyotic lesions have higher mRNA concentrations of prostaglandin E synthase 2 (PTGES2)- the enzyme responsible of PGE2 synthesis- compared to eutopic endometrial cells from those without adenomyosis (59). In a pre-clinical mouse model, prostaglandin D2 genetic deficiency increased endometrial COX2/PTGS2 and PGE2 levels and increased adenomyosis lesion development (60). Therefore, an
imbalance of vasoconstrictor and vasodilator molecules may be involved in the development of adenomyotic lesions but its impact on the development of AUB remains to be determined.

AUB may additionally or alternatively result from aberrant endometrial haemostatic processes, such as fibrin clot formation/degradation ratios. Patients with AUB-E were found to have increased activity of the tissue plasminogen activator (PLAT), compared to those with NMB (61). This mediator activates plasminogen, which is the enzyme responsible for the degradation of fibrin clots. In contrast, the eutopic endometrium and lesions of those with adenomyosis were found to have higher levels of the plasminogen activator inhibitor 1 (SERPINE1) -a PLAT inhibitor-when compared to healthy controls (62). Whether such aberrations result in less fibrinolysis or represent compensatory mechanisms remains to be determined.

Defects in primary haemostasis might also be involved in AUB-A. Tissue factor (F3), a protein involved in the initiation of the coagulation cascade, was found to be increased in the eutopic endometrium as well as in the lesions of adenomyotic patients when compared to healthy controls (63). Interestingly, F3 endometrial immunohistochemical staining of glandular epithelial cells was significantly higher in women with AUB-A than those adenomyosis patients with normal menses (63). To determine the role of haemostasis dysregulation in AUB-A, combining a pre-clinical mouse model of adenomyosis with simulated menstruation (36, 37) would allow genetic and/or pharmacological alteration of the platelet cascade to examine the impact on menstrual blood loss/endometrial repair.

Pathology: Endometrial Repair and Regeneration

Defective vasoconstriction may also affect endometrial hypoxia and menstrual endometrial repair. Patients with AUB-E have been shown to have decreased menstrual endometrial HIF1A protein when compared to those with NMB, as well as a reduction in HIF1 downstream targets (37). In a mouse model of simulated menses, HIF1A genetic deficiency or HIF1 pharmacological inhibition, delayed endometrial repair (37). However, specific cell types driving these HIF1A mediated effects in the menstrual endometrium remain undefined. Those with AUB-E have also been shown to have menstrual deficiencies in other putative endometrial repair factors, with lower protein levels of TGFβ perimenstrually when compared to those with NMB (22).

The decreased bioavailability of HIF1A and TGFβ in patients with AUB-E may affect vascular repair and/or angiogenesis after endometrial shedding (65). Defects in spiral arteriole maturation have been described in AUB. Patients with AUB-E display greater focal discontinuities in endometrial blood vessel walls than those experiencing NMB (66). A lower proliferation rate of vascular smooth muscle cells (VSMCs) in spiral arterioles of those with AUB-E has also been demonstrated and these cells appear critical for vessel integrity and blood flow (67). In addition, VSMCs in the spiral arterioles of AUB-E patients exhibit a lower expression of maturation markers [alpha smooth muscle actin (ACTA2), myosin heavy chain (MYH1)] (68). Moreover, some components of the endometrial endothelial extracellular matrix [laminin (LAM), osteopontin (SPP1), fibronectin (FN1), collagen IV (COL4)] are dysregulated in AUB-E, which may contribute to reduced endothelial vascular integrity (69).

Patients with adenomyosis have also been shown to have evidence of abnormal endometrial vascularisation. The microvascular density of both the eutopic endometrium and adenomyotic lesions was higher than in the endometrium of healthy controls (70, 71). Furthermore, VEGF protein levels show a similar trend, being higher in eutopic endometrium (71, 72) and lesions of adenomyotic patients (52, 70, 73) when compared to healthy controls. None of these studies quantified menstrual blood loss or considered the presence of the symptom of AUB in their analysis. This is necessary to determine if abnormal endometrial vascularisation is causing the symptom of AUB and/or is involved in the development of adenomyotic lesions.

CURRENT TREATMENT

There are a range of medical treatments currently available for the treatment of AUB. This section discusses these options in the context of the menstrual physiological processes on which they exert their actions.

Current Treatment: Endocrine Manipulation

Most current medical treatments for AUB act to override physiological ovarian hormone production. As discussed, however, there are multiple pathways involved in the pathogenesis of AUB-E and AUB-A and endocrine manipulation fails to directly target these processes. Hormonal medication may be more suitable for those with AUB-O where aberrant hormone secretion and regulation leads to AUB symptoms. Despite this, medical therapy in the form of the combined oral contraceptive pill (COCP), oral progestins and levonorgestrel-releasing intrauterine system (LNG-IUS) are commonly offered to patients who present with AUB as first line treatment (74). The COCP has been shown to be effective in reducing HMB and regulating unscheduled bleeding (75) but it is not suitable for those with a history of migraine with aura (sensory disturbance accompanying migraine symptoms), a personal or strong family history of venous thromboembolism, a body mass index (BMI) > 35, smokers over the age of 35 or those wishing to conceive (76). Side effects such as mood changes, skin changes and fluctuation in weight are reported. Oral progestins are available to a larger group of patients, with fewer contraindications, but again these medications come with similar reported side effects and a negative impact on conception which may render them unsuitable for certain populations.

Interestingly, of the hormonal treatments outlined above, only the LNG-IUS has been shown to improve quality of life (77) and there is evidence that it improves HMB (78). A randomised control trial comparing the LNG-IUS and COCPs for symptomatic treatment of adenomyosis, showed the LNG-IUS to be more effective in reducing pain and bleeding (79). However, some women may find IUS insertion painful and the risks of uterine perforation or infection may be unacceptable. It may also cause unpredictable bleeding patterns and hormonal side effects of acne, breast tenderness and mood changes (80).
Gonadotropin releasing hormone (GnRH)-agonists may also be used for AUB management (81, 82). The sustained activation of GnRH receptors leads to their desensitisation, ultimately inhibiting luteinizing hormone (LH) and follicle-stimulating hormone (FSH) synthesis by the pituitary gland. Abrogation of these hormones suppresses ovulation and consequently oestrogen (oestradiol) production. This ovarian hormonal suppression also usually results in the absence of menstruation. However, one of the main downsides of this treatment is the negative side effects caused by oestrogen deficiency. In addition to hot flushes and/or loss of libido, patients may experience a reduction in bone mineral density, increasing the risk of osteoporosis (83). Therefore, its use is limited in younger women. If used in young women, hormone replacement therapy (HRT) is recommended to reduce menopausal symptoms and risk of loss of bone density.

**Current Treatment: Breakdown and Inflammation**

More specific correction of the aberrations present in those experiencing menstrual disorders is currently possible. Non-hormonal medical treatment includes the use of non-steroidal anti-inflammatories (NSAIDs). NSAIDs target the excessive endometrial inflammation observed in AUB-E (48) by inhibiting the COX enzymes that are responsible for the synthesis of prostaglandins (28, 29). The ability of NSAIDs to reduce menstrual blood loss is highlighted in a meta-analysis of 18 randomised controlled trials which demonstrates that NSAIDs are more effective in reducing menstrual blood loss when compared with placebo (84). Mefenamic acid and naproxen have both been shown to result in a reduction in levels of menstrual blood loss, with no significant difference noted between the two (85, 86). NSAIDs use may be limited in patients with a history of gastrointestinal bleeding, inflammatory bowel disease, severe asthma, renal disease, congestive heart failure and cerebrovascular disease. NSAIDs can also affect platelet function and when used in individuals with underlying coagulopathies NSAIDs may be ineffective and may lead to increased bleeding (87).

**Current Treatment: Limiting Blood Loss**

As discussed, a key process involved in limiting menstrual blood loss is primary haemostasis and the creation of a platelet plug. This triggers a series of interactions resulting in conversion of soluble fibrinogen to an insoluble fibrin clot. Tranexamic acid (TXA) is a medication which may be used to reduce the breakdown of the fibrin clot. TXA competitively blocks plasminogen binding sites and reduces the production of plasma and the breakdown of fibrin. In a meta-analysis, which predominantly incorporated results from studies in patients with AUB-E, tranexamic acid was demonstrated to be superior to placebo in reducing menstrual blood loss (88). It has fewer contraindications when compared with hormonal therapies but its use is limited in patients with a history of thromboembolic disease (76).

**EMERGING TREATMENTS**

Current treatment options for AUB remain suboptimal, as highlighted by the results of the Royal College of Obstetricians and Gynaecologists (RCOG) UK HMB audit (89). In this audit of patients attending hospital gynaecology clinics (n = 8183), 37% of women remained “unhappy” or “very unhappy” with their ongoing HMB symptoms (89). There is a clear need for new and improved medical treatment strategies for AUB (Figure 1).

**Emerging Treatments: Endocrine Manipulation**

Ulipristal acetate (UPA) is a selective progesterone receptor modulator with prevailing inhibitory effects on the progesterone receptor that may be used as a treatment for AUB-L (90). The safety and effectiveness of UPA has been assessed in three clinical studies under the PEARL (PGL4001 Efficacy Assessment in Reduction of Symptoms Due to Uterine Leiomyomatata) programme, where UPA administration resulted in effective control of AUB (90). Interestingly, UPA reduces AUB as effectively as the GnRH agonist Leuprorelin but without hypoestrogenic side effects (90). Some adverse effects associated with UPA treatment have been noted, such as weight gain and fatigue and recently potential concerns regarding negative liver effects have been highlighted (91).

The effectiveness of UPA in AUB-L management raises the possibility of trialling this therapeutic strategy in those with AUB-E and AUB-A (Figure 1B). In an observational study in women with adenomyosis, treatment with UPA for 12 weeks reduced symptoms of AUB (92). A phase II, randomised, double-blind controlled trial with UPA 10 mg/day for 3 months in patients with adenomyosis has been registered (NCT02587000). UPA has been shown to impact the endometrium at a cellular and molecular level. In particular, it has been shown to reduce to expression of endometrial steroid metabolising enzymes. A reduction in HSD11B1, known to metabolise cortisol, was observed (93). As previously discussed, cortisol is thought to play a role in regulating the menstrual inflammatory response. These findings support the potential use of UPA in patients with AUB-E.

Aromatase (CYP19A1) has been detected in the endometrium of women with endometriosis, adenomyosis and leiomyomas but not in normal endometrium and has therefore been proposed as a potential therapeutic target (94). A small prospective randomised controlled study compared the oral aromatase inhibitor letrozole and the subcutaneous GnRH agonist Goserein in the treatment of adenomyosis. In both groups, a similar reduction in uterine volume and adenomyoma volume was observed and two patients in the letrozole group became pregnant during treatment (95).

GnRH-antagonists have also emerged as a new strategy for improving AUB (Figure 1B). As opposed to conventional GnRH agonists, GnRH antagonists directly inhibit LH and FSH synthesis. This mechanism of action skips the initial surge of the pituitary hormones aforementioned that occurs with GnRH agonist treatment. Moreover, the dose of GnRH-antagonist used may be titrated to allow only partial suppression of oestrogen. This reduces the potential consequences of a hypoestrogenic state and additional add-back HRT may not be required.
Emerging Treatments: Breakdown and Inflammation
As discussed above, inflammation and NFκB signalling pathways may play a crucial role in the initiation of endometrial breakdown, with overactivation resulting in AUB (Figure 1B). Andrographolide is an active ingredient from the plant *Andrographis paniculata* and has been used for many years in traditional Chinese medicine for the treatment of inflammatory disorders. It has been shown to suppress NFκB activation (97). In a pre-clinical model of adenomyosis, the intragastric administration of andrographolide reduced myometrial leukocyte infiltration as well as adenomyosis-derived pain (98). The impact of andrographolide treatment on menstrual blood loss was not reported in this study and merits further examination.

A more refined strategy may be to target downstream mediators of NFκB that have been shown to be dysregulated in AUB, such as TNF (48). At present, there is a wide range of anti-TNF antibodies which effectively block the biological function of
TNF (99). However, systemic administration of these drugs is not without side effects, given the key role of TNF against pathogen invasion (99). Therefore, local endometrial anti-TNF delivery may be beneficial. As an example, polymer-coated nanoparticles containing small interfering (si)RNA targeting TNF have been proven to be successful in a mouse model of rheumatoid arthritis, reducing TNF levels in serum and arthritic joints (100). This technology has the potential to be adapted for endometrial disorders once the endometrial source of TNF is confirmed (Figure 1A).

Cortisol deficiency may also play a key role in the development of AUB-E via dysregulation of the endometrial repair processes. The role of low dose dexamethasone as a treatment to reduce HMB has been demonstrated in a recent response-adaptive randomised placebo-controlled dose-finding parallel group trial (DexFEM) (101). This trial showed that dexamethasone 1.8 mg once daily over 5 days in the mid-secretory phase reduced menstrual blood loss when compared with placebo. However, 75% of the participants in the dexamethasone group reported adverse events, compared with 58% of those taking placebo. Nonetheless, this study demonstrated that dexamethasone may provide an effective treatment option for AUB in women who wish to avoid ovarian hormone based treatments (Figure 1A).

Emerging Treatments: Limiting Blood Loss
Treatments that correct aberrations identified in the endometrial coagulation system of those with AUB have potential as novel therapeutics or preventative strategies for AUB-A (Figure 1B). In a mouse model of adenomyosis, treatment with Ozagrel, a platelet aggregation inhibitor, supressed myometrial infiltration and improved adenomyosis related pain (102). As highlighted by the authors of this proof-of-concept study, concerns remain about the possible risk of haemorrhage associated with such an anti-platelet therapy. While this study adds to our understanding of the role of activated platelets in the pathogenesis of adenomyosis, further examination of the safety profile of such medications is required before translation to clinical trials.

Emerging Treatments: Endometrial Repair and Regeneration
Markers of hypoxia have been detected in the human endometrium at menstruation (38) and physiological hypoxia has been shown to be important in endometrial repair during simulated menses in the mouse (37). Pharmacological stabilisation of HIF1 in a mouse model of delayed endometrial repair rescued the phenotype and improved repair, indicating that targeting the hypoxia pathway may be a valid approach in the treatment of AUB (37) (Figure 1A). HIF1A stabilisation is not an unfamiliar therapeutic strategy in the treatment of non-gynaecological disorders. Pharmacological stabilisation of HIF1A has proven to be effective and safe in the treatment of anaemia in chronic kidney disease (103, 104). In addition, a small HIF1A stabiliser has showed promise in accelerating diabetic wound healing in different pre-clinical models (105). These compounds may have therapeutic benefits in the endometrium, which is amenable to local and intermittent treatment.

Correcting the partial deficiency of TGFB during menstruation that has been detected in the endometrium of those with AUB-E may present another valid therapeutic strategy for AUB (Figure 1A). Due to the numerous pleiotropic effects of TGFB as a cytokine, the systemic administration of a soluble version is far from ideal (106). However, recent literature offers different strategies for local delivery of soluble mediators. Using inert biodegradable nanoparticles loaded with TGFB, McHugh et al. achieved T cell-specific delivery both in vitro and in animal models (107). This technique has the potential to be applied in the endometrial environment, perhaps via transvaginal administration, targeting endometrial, immune and/or vascular endothelial cells. Liposomes are another alternative nanoparticle with good pre-clinical results (108) that could be of use in TGFB delivery if designed for local endometrial action.

A FUTURE MODEL FOR MANAGEMENT OF AUB

Despite advances in understanding of the mechanisms and pathological processes that lead to AUB, many treatments remain broad spectrum and generic. Often the focus is on AUB symptom control rather than specific diagnosis and targeted treatment.

Achieving an accurate diagnosis starts with focused history taking and clinical examination and should involve reference to FIGO AUB System 1 (nomenclature) and System 2 (classification; PALM-COEIN) (1). This directs further relevant investigations and personalises management. For example, identifying and understanding a patient’s wish for fertility, previous experience with treatments and assessment of the size, position, regularity, mobility, and tenderness of the uterus will help aid clinicians to tailor management options specifically suited to that patient and their diagnosis. Further research to determine how patient demographics such as age, BMI and physical activity influence menstrual blood loss and response to treatment may also help to select more effective treatments for individuals (109).

To assist clinical diagnosis, bedside tests that identify the presence of structural uterine disorders and/or identify the specific cause(s) of endometrial dysfunction would be highly valuable. For example, the ability to identify if a women with AUB-E had aberrations in endometrial hypoxia, inflammation and/or coagulation at the time of endometrial sampling would facilitate personalised medicine and correction of the specific underlying defect causing AUB. These tests may also assist in the selection of appropriate investigations such as ultrasound or hysteroscopy.

The gold standard for diagnosis of adenomyosis is histopathological confirmation of eutopic endometrium within the myometrium. However, the use of transvaginal ultrasound (TVUS) in achieving accurate diagnosis of adenomyosis is highlighted in a systematic review (46). Of the 8 studies included, TVUS 2D and TVUS 3D were shown to be effective methods for diagnosis of adenomyosis with pooled sensitivity of 84 and 89%, and pooled specificity of 64 and 56%, respectively (46). These findings support the use of imaging in obtaining an accurate
diagnosis but focused research to improve the specificity of such methods would have significant clinical and scientific benefits.

Several biomarkers for adenomyosis have also been proposed. For example, proteomic analysis has shown that moesin, a cytoskeletal adaptor protein, is higher in the endometrium of those with adenomyosis vs. controls (110). This finding may also help our understanding of the development of adenomyosis as moesin expression is correlated with the extent of invasiveness seen in some tumours, such as gastric adenocarcinoma (110). Full discussion of all emerging biomarkers for AUB is not possible in this review but such findings highlight the potential for improving the non-invasive diagnosis of this debilitating symptom.

Improved diagnosis of structural and non-structural causes of AUB will not only direct treatment but will facilitate research into the pathogenesis of specific diagnoses (e.g., AUB-E/A) and reveal new therapeutic targets and preventative strategies to improve the lives of those who suffer from AUB.

CONCLUSIONS

Furthering our understanding of menstrual physiology confirms that there is a complex interplay of endocrine, immune, haemostatic and vascular regulatory functions. This complexity is mirrored in the pathological aberrations that may occur in each of these pathways, resulting in AUB. We have demonstrated that current medical management of AUB is suboptimal by highlighting poor satisfaction rates and the lack of specific, targeted treatments. Emerging treatments offer the promise of more specific targeting of the underlying pathology causing AUB-E and AUB-A. Accurate diagnosis of the underlying cause of AUB should be a clinical and research priority. Future research should also focus on developing therapies which have direct actions against the pathological processes which have been demonstrated to result in AUB-E and AUB-A. Focused clinical assessment and imaging techniques may help toward this goal but the development of non-invasive biomarkers would be a significant step toward improving management. These efforts would facilitate the development of personalised, effective, acceptable treatments to improve the lives of those who experience AUB.

AUTHOR CONTRIBUTIONS

MW and RM-A wrote the manuscript. JM, MW, and RM-A planned and edited the manuscript. All authors have read and approved the final version of this manuscript.

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