Expression of vascular endothelial growth factors and their receptors in human endometrium from women experiencing abnormal bleeding patterns after prolonged use of a levonorgestrel-releasing intrauterine system

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BACKGROUND: Menstrual bleeding disturbances are a common initial complaint among users of the levonorgestrel-releasing intrauterine system (LNG-IUS). In this study, women who experienced bleeding disturbances recurring after a previous period of problem-free use and who therefore wanted removal of their LNG-IUD were investigated. Vascular endothelial growth factors (VEGFs) and their receptors are thought to be involved in normal endometrial angiogenesis. The aim of the study was to elucidate the possible association of these VEGF and receptors with bleeding disturbances among users of LNG-IUS. METHODS: Endometrial biopsies were obtained from users of the LNG-IUS who complained of bleeding disturbances (n = 17) and from women without such problems (n = 14). The endometrial expression of these VEGFs and their receptors was analysed using immunohistochemistry. RESULTS: Endometrial endothelial cells from LNG-IUS users with menstrual bleeding disturbances exhibited significantly higher immunoreactivity for VEGFR-1 and VEGFR-3 than those from women without bleeding disturbances. Stromal cells showed significantly lower immunoreactivity for VEGF-A in samples from LNG-IUS users with bleeding disturbances than in those without. CONCLUSION: Changes in the expression of these angiogenic growth factors and their receptors in LNG-IUS-exposed endometrium might be involved in the formation of fragile and dysfunctional blood vessels that subsequently give rise to bleeding disturbances.

Key words: angiogenesis/endometrium/levonorgestrel-releasing intrauterine system/menstrual bleeding disturbances/vascular endothelial growth factors

Introduction

The levonorgestrel-releasing intrauterine system (LNG-IUS; Schering AG, Germany) is a widely used contraceptive method (Odlind, 1998), which is also used for the treatment of menorrhagia and for endometrial protection during HRT (Andersson and Rybo, 1990; Wollter-Svensson et al., 1997). While the LNG-IUS is highly effective, safe, long-acting and reversible and associated with a pronounced reduction of menstrual blood loss, bleeding disturbances are frequent complaints, particularly during the first 3–6 months of use (Odlind and Fraser, 1990; Andersson et al., 1994). Thus, initial bleeding problems, mainly frequent, irregular episodes of spotting/bleeding, are important reasons for discontinuing LNG-IUS use and have been reported by ~14% of users, whereas prolonged or heavy bleeding is a rare reason for its removal (Andersson et al., 1994; Rönnerdag and Odlind, 1999). The rate of irregular bleeding/spotting decreases and the rate of amenorrhoea increases with increasing duration of LNG-IUS use, amenorrhoea being reported among ~20% of long-term users (Rönnerdag and Odlind, 1999). Amenorrhoea is often well tolerated if the woman is carefully counselled and therefore a rare reason for removal (Backman et al., 2000). Although abnormal bleeding patterns after prolonged (>6 months) use of the LNG-IUS are uncommon, some complaints about unexpected bleeding problems occurring after prolonged use have been reported anecdotally by clinic staff. The mechanisms underlying these bleeding disturbances remain unclear.

The main mechanisms of action of LNG-IUS, as well as its most common side-effects, are based on profound local effects on the endometrial morphology and function exerted by levonorgestrel released from the LNG-IUS (Nilsson et al., 1984; Barbosa et al., 1995; Gu et al., 1995; Pakarinen et al., 1998). Endometrium exposed to intrauterine delivery of progestosterone or progestogen also show profound changes in its vasculature with a substantially decreased number of spiral arterioles, a significantly reduced density of normal capillaries, and some large thin-walled venule-like blood vessels (Zhu et al., 1989; Gu et al., 1995; Hickey et al., 1998; Runic et al., 2000). It is not known why these thin-walled

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venule-like blood vessels develop, and their significance as a cause of the bleeding disturbances has not been established, but they are strong candidates as a causative factor. Previous data have indicated that the morphology of the endometrial vasculature in users of LNG-IUS is similar to that of the endometrium in users of the Norplant® implants, a levonorgestrel-releasing contraceptive method that is associated with more frequent bleeding problems than the LNG-IUS (Rogers, 1996; Hague et al., 2002). Some of the reported changes in the endometrial vasculature probably account for the development of bleeding disturbances among some users of LNG-IUS. Moreover, levonorgestrel itself has been shown to alter the angiogenic activity (Hague et al., 2002), but the significance of this remains unknown. Morphological and functional changes in the endometrial vasculature are regulated by stimulators, inhibitors and modulators of angiogenesis (Smith, 2001). The above findings together indicate that prolonged or recurrent bleeding disorders among users of LNG-IUS could be caused by a disturbance in the regulation of angiogenesis or blood vessel function.

Endometrial repair and growth require neo-vascularization, which occurs by angiogenesis, a process whereby new capillaries are formed from existing vessels (Folkman and Klagsbrun, 1987; Rogers and Gargett, 1998; Smith, 2001; Gambino et al., 2002). The precise molecular and cellular mechanisms that mediate these angiogenic processes are not fully understood, but they are most probably related to cyclic changes in circulating levels of ovarian steroids and locally produced factors (Giudice, 1994). Most probably there is an expression of estrogen receptor beta in endometrial endothelial cells (Critchley et al., 2001; Lecce et al., 2001) whereas the expression of progesterone receptors is more controversial since they have only been demonstrated in cultured endometrial endothelial cells (Iruela-Arispe et al., 1999). A high endometrial concentration of levonorgestrel, however, reduces the levels of these receptors (Zhu et al., 1999), thus diminishing the possibility of endometrial support by estrogen and progesterone. This will probably affect the expression of locally produced angiogenic growth factors and their receptors, causing local abnormalities in the vascular structure and/or function, which may then lead to bleeding disturbances in some women.

Vascular endothelial growth factors (VEGF) and their receptors are believed to be required for the control of endometrial angiogenesis, and probably are key factors in the occurrence of abnormal uterine bleeding (Smith, 1998). VEGF-A, B, C and D have proved to be mitogenic for endothelial cells in vitro, and can induce angiogenesis in vivo (Ferrara and Henzel, 1989; Joukov et al., 1996; Lee et al., 1996; Olofsson et al., 1996; Yamada et al., 1997; Achen et al., 1998). The different VEGFs bind to one or two of the three VEGF receptors (VEGFR): VEGF-A binds to VEGFR-1 and VEGFR-2; VEGF-B binds to VEGFR-1; and VEGF-C and VEGF-D each binds to VEGFR-2 and VEGFR-3 (de Vries et al., 1992; Quinn et al., 1993; Achen et al., 1998).

Quite recently it was reported that LNG-IUS altered the endometrial expression of the angiogenic growth factors VEGF-A, transforming growth factor (TGF)-β1 and fibroblast growth factor (FGF)-2 and that there was a positive correlation between endometrial VEGF levels and the number of bleeding/spotting days (Roopa et al., 2003). It has also been shown that the expression of VEGF-A is significantly reduced in both stroma and glands after 3 months use of LNG-IUS (Laoag-Fernández et al., 2003).

The aim of this study was to elucidate the possible association of VEGF-A, B, C and D and their receptors with bleeding disturbances among users of LNG-IUS by comparing endometrium from women who complained of bleeding disturbances appearing after ≥6 months of successful use of the LNG-IUS with endometrium from women using the LNG-IUS without such problems.

### Materials and methods

**Recruitment of women and collection of samples**

Seventeen current users of the LNG-IUS, who complained of abnormal bleeding patterns after ≥6 months of use and were therefore considering removal of the LNG-IUS, were recruited among patients attending family planning clinics. Seventeen women who had used their current LNG-IUS without having experienced abnormal bleeding pattern after ≥6 months and wanted to continue its use, were recruited as controls through advertisements in the local newspaper.

All were using the LNG-IUS for contraception. Each woman with a bleeding problem was matched with a control woman with regard to age (±1 year) and duration of use of the current LNG-IUS (±3 months) (Table I). The women were healthy and without medication.

Bleeding patterns were evaluated by means of a bleeding diary. For a woman to be eligible for the study, i.e. allocated to the group with bleeding disturbances, she should have had at least one episode of bleeding/spotting lasting >7 days or at least two bleeding/spotting episodes during the preceding 28 day period, either symptom occurring after ≥6 months of LNG-IUS use without such problems (Table I). In addition, she should request removal of the LNG-IUS due to this problem. For a user of LNG-IUS to be eligible as a control, the duration of any bleeding/spotting episode should have been

<table>
<thead>
<tr>
<th>Variable</th>
<th>With bleeding disturbances (n = 17)</th>
<th>Without bleeding disturbances (n = 14)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>37.1 (5.3)</td>
<td>38.1 (5.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of use of the current LNG-IUS (months)</td>
<td>39.0 (16–51)</td>
<td>40.0 (14–51)</td>
<td>NS</td>
</tr>
<tr>
<td>No. of bleeding days/28 day assessment period</td>
<td>23.6 ± 3.1</td>
<td>3.2 ± 1.0</td>
<td>0.00001</td>
</tr>
<tr>
<td>No. of bleeding episodes/28 day assessment period</td>
<td>3.9 ± 0.5</td>
<td>1.1 ± 0.3</td>
<td>0.00001</td>
</tr>
<tr>
<td>Duration of the current bleeding problems (months)</td>
<td>9.0 (3–18)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Serum estradiol (pmol/l)</td>
<td>275 ± 71</td>
<td>185 ± 41</td>
<td>NS</td>
</tr>
<tr>
<td>Serum progesterone (nmol/l)</td>
<td>6.8 ± 2.3</td>
<td>7.9 ± 3.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

The values are presented as mean ± SEM. Statistical differences in the concentration of sex steroids and the number of bleeding days and episodes between the two groups were calculated with the Mann–Whitney U-test. LNG-IUS = levonorgestrel-releasing intrauterine system; NS = not significant.
<7 days and there should have been no more than one bleeding/spotting episode during the 28 day bleeding assessment period (Table I).

An endometrial biopsy specimen was collected by blind curettage of the anterior and posterior aspects of the corpus part of the uterine cavity after removal of the LNG-IUS. The biopsies were collected in the late luteal phase in the control women and in those women with abnormal bleeding patterns in whom the luteal phase could be defined (Table I). Women who so wished had a new LNG-IUS inserted immediately after the biopsy had been taken. Unfortunately, three of the patients in the control group had to be excluded due to poor quality of the endometrial biopsies. Samples were immediately fixed in 4% neutral buffered formalin at +4°C overnight, and routinely embedded in paraffin. Sections 5 mm thick were cut for immunohistochemistry and routine haematoxylin and eosin staining. Prior to the biopsies a venous blood sample was taken for assay of serum levels of estradiol and progesterone. Hormone analyses were performed with a method based on enzyme-amplified chemiluminescence (Immulite®; Diagnostic Products Corporation, USA) in accordance with the supplier’s instructions. Ethical approval of the study was obtained from the Ethics Committee of Uppsala University and the women gave their informed consent prior to all tissue sampling.

**Immunohistochemistry**

The following primary antibodies were purchased from Santa Cruz Biotechnology Inc., USA: mouse monoclonal anti-VEGF-A (sc-7269) and anti-VEGFR-2 (sc-6251); goat polyclonal anti-VEGF-B (sc-1878), anti-VEGF-C (sc-1881), and anti-VEGF-D (sc-7602); and rabbit polyclonal anti-VEGFR-1 (sc-316). The monoclonal anti-human VEGFR-3 has been described by Jussila et al. (1998) and was a gift from Professor Kari Alitalo. Monoclonal anti-CD31 antibody (PECAM-1 M0823) was purchased from Dako A/S, Denmark. All secondary antibodies [anti-mouse (BA-2000), anti-goat (BA-9500) and anti-rabbit (BA-1000)] were purchased from Vector Laboratories Inc., USA.

The streptavidin–biotin technique was used for immunohistochemistry. All incubations were performed at room temperature unless otherwise stated. The sections were deparaffinized in xylol and then rehydrated in a graded series of ethanol according to standard procedures. Antigen retrieval was done by microwave treatment in sodium citrate buffer (10 mmol/l, pH 6.0; Zymed Laboratories, Inc.) containing bovine serum albumin and biotin blocking solution (Blocking Kit; Vector Laboratories Inc., USA) for 20 min. Endogenous peroxidase activity was blocked by treatment in sodium citrate buffer (10 mmol/l, pH 6.0; Zymed Laboratories, Inc.) containing 0.3% hydrogen peroxide. The sections were then incubated with the appropriate primary antibody (diluted 1:200) overnight, and endogenous avidin and biotin binding sites were blocked by incubation with avidin and biotin blocking solution (Blocking Kit; Vector Laboratories, USA) for 30 min. Non-specific antibody binding was reduced by diluting the primary and secondary antibodies in antibody diluent solution (Zymed Laboratories, Inc.) containing bovine serum albumin. Sections were incubated with primary antibodies overnight at +4°C: antibodies to CD31 (0.7 µg/ml), VEGF-A (2.0 µg/ml), VEGF-B (0.4 µg/ml), VEGF-C (0.5 µg/ml), VEGF-D (0.5 µg/ml), VEGFR-1 (0.1 µg/ml), VEGFR-2 (0.2 µg/ml) and VEGFR-3 (0.7 µg/ml). The sections were then incubated with the appropriate biotinylated secondary antibody diluted 1:200 for 45 min prior to incubation with horseradish peroxidase–streptavidin (1:200, SA-5004; Vector Laboratories, Inc.) for 45 min. The antigen–antibody reaction was visualized using 3,3’-diaminobenzidine as chromogen (DAB, S-300010; Dako, A/S). After washing, the sections were counterstained with Mayer’s haematoxylin, dehydrated, and mounted in mounting medium (Perix®; Histolab products AB, Sweden).

Positive controls were performed for all antibodies by staining tissues with known expression of the corresponding epitopes (human placenta and human lymph node). Negative controls were performed by replacing the primary antibodies with non-immune rabbit immunoglobulin (X 0903; Dako, A/S), isotype-matched irrelevant monoclonal mouse IgG (X 0931; Dako, A/S), or normal goat serum (X 0907; Dako, A/S) at the same concentrations as those of the corresponding primary antibodies. Inter-assay variability in immunoreactivity was assessed by staining a section from the same paraffin block in every assay.

**Scoring analysis of immunoreactivity**

As immunohistochemistry is a more qualitative than quantitative method, the stained areas of the blood vessels, stromal compartment and epithelial cells were estimated instead of the staining intensity. An area was considered as positive when the staining intensity was stronger than that found in the corresponding negative control. One person evaluated the immunohistochemical staining blindly as to which group (i.e. LNG-IUS without or LNG-IUS with bleeding disturbances) the endometrial sample originated from. Ten randomly chosen areas of the functionalis layer of the endometrium, shown on a computer screen via a digital camera connected to the microscope, were evaluated on two occasions. A score was assigned semi-quantitatively on a 4-point scale from 0 to 3, where 0 = no staining, 1 = less than one-third of the area stained, 2 = more than one-third but less than two-thirds stained, and 3 = more than two-thirds stained. Every compartment (i.e. blood vessels, stromal cells and epithelial cells) was counted separately and the scores from every woman were summed and the means were calculated.

**Blood vessel counting**

A single observer blinded to the sample background counted the number of CD31-positive vessels in at least five randomly chosen fields in the functionalis of the endometrial specimens by using computer-assisted picture grabbing. The results were averaged and calculated as blood vessels per mm².

**Statistical analysis**

Differences between the two groups (i.e. endometrium from users of LNG-IUS with and without menstrual bleeding disturbances), were considered significant at P < 0.05. Distributions of measured values of the variables were calculated by the Shapiro–Wilk test. The normally distributed variables underwent an overall one-way analysis of variance (ANOVA) to predict significant differences between the groups. If significance was found in a variable, pairwise one-way ANOVA tests were run. When variables were not normally distributed, the Kruskal–Wallis test was used for an overall comparison to predict significant differences between the groups. When possible significance was detected, the Mann–Whitney test was used between the groups. Results for the blood vessel density and amount of staining are presented as mean ± SEM.

**Results**

Immunohistochemical control stainings were as expected for positive controls, and no immunostaining was seen in negative controls when non-immune goat serum, irrelevant rabbit immunoglobulin, or mouse IgG was used instead of the corresponding primary antibodies. The numbers of bleeding episodes and days during the 28 day assessment period differed significantly between the two groups of patients (Table I). The serum levels of estradiol and progesterone did not differ significantly between the two groups (Table I).
Immunoreactivity of VEGF ligands and receptors in endothelial cells

VEGF-A–D and VEGFR-1–3 were present to various degrees in all endometrial samples (Figures 1 and 2). In the tissue sections, the endothelial layer of endometrial blood vessels was often disrupted or even absent. The immunostaining of the endothelial layer was fairly uneven. There were no significant differences in immunoreactivity for the VEGF ligands between the two groups of women. However, the LNG-IUS users without bleeding disturbances exhibited significantly lower immunoreactivity for VEGFR-1 and VEGFR-3 than those with such disturbances. Thus, among

Figure 1. Immunohistochemical stainings of vascular endothelial growth factors (VEGFs) and their receptors in serial sections of endometrium from a woman after 17 months use of a levonorgestrel-releasing intrauterine system, with no abnormal bleeding (a–h), and from a woman with recurrence of bleeding disturbances (i–p). The sections were stained for (a, i) VEGF-A, (b, j) VEGF-B, (c, k) VEGF-C, (d, l) VEGF-D, (e, m) VEGFR-1, (f, n) VEGFR-2, (g, o) VEGFR-3 and (h, p) CD31. Scale bars = 10 μm.
users of LNG-IUS, the endometrial blood vessels showed differences in the expression of VEGFR-1 and VEGFR-3 between those with and those without abnormal bleeding patterns.

**Immunoreactivity of VEGF ligands and receptors in stromal cells**

The stroma exhibited significant differences in immunoreactivity for VEGF-A, but not for the other studied ligands and receptors, between the LNG-IUS users with and without abnormal bleeding patterns. The pattern of immunoreactivity varied among the studied VEGF ligands and receptors. VEGF-A was present in some prominently stained cells, probably lymphoid cells, which regularly seemed to express all studied VEGF ligands and receptors. VEGF-A showed significantly more immunoreactivity in LNG-IUS users without abnormal bleeding than in those with such bleeding. The staining for VEGF-B was generally seen in the cytoplasm, closely associated with the stromal cell nuclei, while the staining for VEGF-C was usually confined to single cells or clusters of cells. The observed differences in immunostaining of VEGF-B and VEGF-C between the two groups were not significant.

**Immunoreactivity of VEGF ligands and receptors in epithelial cells**

The epithelial cells displayed a typical progesterone-affected appearance, i.e. uneven and fairly flat. The luminal epithelium, in particular, was in some samples barely microscopically visible. The immunoreactivity for VEGF-C was significantly higher in LNG-IUS users without abnormal bleeding than in those with abnormal bleeding. In contrast, VEGFR-1 showed significantly lower immunoreactivity in the former group than in the latter.

**Blood vessel density**

The mean number of blood vessels per mm² ± SEM was 180 ± 12 in endometrium of users of LNG-IUS without abnormal bleeding and 195 ± 15 in users of LNG-IUS with abnormal menstrual bleeding. This difference was not significant.

**Serum concentrations of estradiol and progesterone**

The serum concentrations of estradiol and progesterone in the two groups are presented in Table I as mean ± SEM. The differences between the groups were not significant.

**Discussion**

VEGF ligands and receptors have been shown to be involved in several steps of the regulation of angiogenesis and blood vessel function (Hanahan, 1997). We have previously shown that VEGF-A–C and VEGFR-1–3 are probably important for the normal angiogenesis in the human endometrium (Möller et al., 2001, 2002). These growth factors and receptors may also be involved in the pathogenesis of the bleeding disturbances seen in some users of LNG-IUS. VEGF-D was included in the current study since it has features in common with VEGF-C (Yamada et al., 1997; Achen et al., 1998). The only member of the VEGF family that has previously been studied in endometrium from users of LNG-IUS is VEGF-A (Charnock-Jones et al., 2000; Hague et al., 2002; Laoag-Fernandez et al., 2003; Roopa et al., 2003). It has recently been reported that the use of LNG-IUS changes the endometrial expression of VEGF-A, TGF-β1 and FGF-2 and that there seems to be a positive correlation between the endometrial VEGF-A content and the number of bleeding/spotting days (Roopa et al., 2003). The current study was designed to compare endometrium from LNG-IUS users with and without bleeding disturbances. Among users of LNG-IUS we found that the expression of several VEGF ligands and receptors differed between those with and those without bleeding disturbances, data that might add new information about the aetiology of LNG-IUS-induced bleeding disturbances. The immunoreactivity was analysed by scoring the stained area but not the staining intensities. This unusual approach was adopted since the staining intensities in adjacent areas, for instance in adjacent blood vessels, differed considerably, making it difficult to decide a definitive score for staining.
intensity. As immunohistochemistry is a more qualitative than quantitative method, we decided that scoring of the presence of the epitope expressed as stained area of a certain endometrial compartment might be more correct than trying to base the results on inexact staining intensities. A similar scoring method has previously been used by others (Yokoyama et al., 2003).

Blood vessels
It is interesting to note that the blood vessels, which are the target tissue for angiogenic stimuli, showed differences between the two groups of women in their expression of VEGF receptors but not in that of VEGF ligands, while the surrounding stroma displayed an opposite pattern. It has previously been suggested that the expression of receptors in the endothelial cells might be more important for the regulation of angiogenic activity than that of their ligands (Möller et al., 2001). This possibility is supported by the current observations.

Stromal cells
It is still uncertain how estradiol and progesterone regulate the endometrial expression of VEGF-A, but it is known that the promoter region of the VEGF-A gene contains an estrogen response element but no progesterone response element (Tischer et al., 1991). This does not, however, explain the difference in stromal cell expression of VEGF-A between users of LNG-IUS with and without bleeding disturbances, but it might possibly be connected with the opposite pattern of expression of VEGFR-1 in endothelial cells. This co-variation in expression of VEGF-A and VEGFR-1 between users of LNG-IUS with and without menstrual bleeding disturbances might therefore be involved in the pathogenesis of bleeding disturbances in users of LNG-IUS.

Epithelial cells
The finding of a significantly lower expression of VEGF-C and significantly higher expression of VEGFR-1 in users of LNG-IUS with bleeding disturbances than in those without are difficult to interpret. Endometrial epithelial cells have previously been shown to express VEGF and VEGF receptors (Krüssel et al., 1999; Möller et al., 2001, 2002). What function they might have at non-vascular sites is not known, but it has been shown that VEGF-A stimulates expression of inositol phosphate in mouse kidney epithelial cells (Senthil et al., 2002). Epithelial cells might also release VEGF into the stroma to maintain sufficient angiogenic activity for the formation of the sub-epithelial capillary plexus during the early secretory phase of the menstrual cycle. It is also possible that VEGF are secreted into the lumen of the uterus. VEGF-C has been shown to be expressed in nasal epithelium (Saaristo et al., 2000), which further supports the idea that this growth factor is involved in inflammatory processes and not only in the regulation of angiogenesis and lymphangiogenesis. These examples of actions of VEGF in epithelial cells imply that they might have some other function in addition to the generally accepted one, such as facilitating proliferation and differentiation of endometrial glandular epithelial cells.

Blood vessel density
It is not surprising that there were no differences in blood vessel density between the two groups of patients since they both have received the same treatment regimen. Also, there is still no generally accepted consensus regarding the angiogenic activity and the microvascular density in endometrium from women treated with progestogens. Some previous studies have shown that users of Norplant® show decreased endothelial cell proliferation and an increased microvascular density (Johannisson, 1990; Rogers et al., 1993), while in others a decreased endometrial vascular density has been found in women taking high-dose orally administered progestogens (Song et al., 1995). A possible explanation for these discrepancies is that different routes of drug administration result in highly different endometrial concentrations of the progestogen and thereby differences in the angiogenic activity and microvascular density in the endometrium.

Study design
Women complaining of bleeding pattern disturbances with the LNG-IUS are heterogeneous. By creating pairs with a woman with bleeding abnormality matched by age and duration of LNG-IUS use with a control woman, the study design tried to establish groups that were comparable, despite this heterogeneity.

Bleeding pattern with the LNG-IUS
In this study, abnormal bleeding was defined as bleeding lasting >7 days or at least two episodes of bleeding during the preceding 28 day assessment period. This is different from the common definition with other long-acting progestogen-only contraception, i.e. injectables and implants, where a prolonged bleeding is defined as lasting ≥10 days and frequent bleeding is defined as more than three episodes during a 90 day period (Belsey, 1991). However, those methods, which also cause inhibition of ovulation, are often associated with long periods of irregular, frequent or continuing bleeding, whereas the ‘normal’ bleeding pattern with the LNG-IUS, which does not usually inhibit ovulation, after an initial period of 3–6 months, is characterized by short, scanty and regular or infrequent bleeding episodes (Suvisaari and Lahteenmaki, 1996). Thus, the bleeding pattern during LNG-IUS use is expected to gradually transform towards a state of scanty bleeds or amenorrhoea and the recurrence of heavy, prolonged or frequent bleeding is considered as abnormal for that particular method. None of the women in the study complained of heavy bleeding, but their bleeding patterns that caused them to want removal of their LNG-IUS differed considerably from the expected bleeding pattern with LNG-IUS. We do not believe that we have used a novel classification system but rather applied a simplified version of the Belsey classification, which appeared appropriate for the purpose of the present study. A reference period of 28 days is also completely in line with the proposal of Belsey (1988).
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