 doi:10.20944/preprints201912.0012.v1

Biomarker validation of a new case definition of menstrual cycle-associated syndrome (MCAS)

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 doi:10.20944/preprints201912.0012.v1

Abstract

Premenstrual syndrome (PMS) frequently occurs in women of childbearing age. There are different case definitions of PMS, one proposed by the American College of Obstetricians and Gynecologists (ACOG) and another based on the Daily Record of Severity of Problems (DRSP) scores. Here we review our recent papers indicating that the discovery of biomarkers of menstrual cycle-related symptoms is strongly dependent on the case definitions used and that the gold standard methods used to asses PMS, including the ACOG case definition, induce a high degree of false-negative findings. We propose a new case definition of the menstrual cycle-associated syndrome (MCAS), which is characterized by increased DRSP scores during the menstrual cycle and additionally by an exaggerated increase in symptoms the week prior to the menses. This case definition performed well and was externally validated by diverse biomarkers including plasma levels of progesterone and estradiol, chemokines (e.g. CCL2, CCL5 and CCL11), epidermal growth factor, hydroperoxides, paraoxonase 1 activity and complement C4. In conclusion, when evaluating menstrual cycle-related symptoms and their associations with biomarkers, we propose to assess daily measurements of the DRSP and based on those scores to a) use the diagnosis of MCAS as an indicant of menstrual cycle-related symptoms; and b) examine the associations of the time series in the DRSP and its subdomains (e.g. depression, physio-somatic, anxiety) and those in biomarkers including distributed lag models.

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Key words: premenstrual, depression, inflammation, neuro-immune, oxidative stress, antioxidants

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Introduction

Premenstrual syndrome (PMS) and the classical diagnosis of PMS

Premenstrual syndrome (PMS) is characterized by recurrent physical, emotional, and behavioral symptoms which develop during the second half (luteal phase) of the menstrual cycle and disappear within a few days after menstruation [1]. PMS occurs in 50–80% of reproductive-age women ranged from mild to severe. The symptoms of PMS comprise irritability, sadness, depression, mood swings, decreased concentration, sleep problems, fatigue, bloating, and breast discomfort, which all together may disturb abilities of these women to function normally [2].

Different case definitions are used to make the diagnosis of PMS. A first is based on assessments of the Daily Record of Severity of Problems (DRSP) [3], which was developed as a tool to screen for DSM-IV criteria for Premenstrual Dysphoric Disorder (PMDD)[3]. The DRSP is useful in women who are suspected to have PMS to self-rate the severity of symptoms associated with PMS [3] and to confirm the appearance of symptoms during the luteal phase and the normalization of those symptoms within a few days after menses and the follicular phase. Many studies have applied the DRSP to make the diagnosis of PMS for example by using a) a cutoff value of 50 on the first day of menses to screen for premenstrual disorders [3-5], and b) a DRSP score of at least 70 on day -5 to -1 of the cycle coupled with a difference of at least 30% between the premenstrual (day -5 to -1) and postmenstrual (day 6-10) DRSP score to diagnose PMS [6].

Another case definition of PMS, which is widely used in clinical settings, is that proposed by the American College of Obstetricians and Gynecologists (ACOG) [7]. ACOG criteria consider PMS when one or more affective and physical symptoms are present 5 days prior to the menses and when symptomatic remission occurs within 4 days after the onset of menses without symptom recurrence until at least day 13 of the next cycle. This pattern should

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occur for at least 3 consecutive menstrual cycles with significant dysfunctions in social, academic, or work performance during the symptomatic phase [7].

New case definitions

Recently, we investigated the alterations in the DRSP scores, which were assessed on a daily basis, in 41 women namely 21 with and 20 without increased PMS symptoms as assessed using a DRSP score of 50 on the first day of the menses [3-5]. We used 4 time points during the menstrual cycle to examine associations with biomarkers, namely day 7 (T1), day 14 (T2), day 21 (T3) and day 28 (T4) of the menstrual cycle. The duration of a normal menstrual cycle is 28 days with a range of 26-35 days whereby T1 DRSP values represent the mid-follicular phase symptom severity when estrogen levels are rising. T2 represents the time of ovulation or mid-cycle values when there is a decline in estrogen levels. T3 represents the mid-luteal phase symptom severity when progesterone levels reach their peak. T4 represents the end of the cycle when all hormones levels decline to their baseline levels [8-10].

Based on the inspection of the variations of the DRSP values across the menstrual cycle, we decided to construct 2 new case definitions of menstrual-cycle related symptoms, a first reflecting increased DRSP values during the peri-menstrual period named Pre-Menstrual Syndrome (PeriMS) and a second reflecting increased DRSP values all over the menstrual cycle named Menstrual Cycle Associated Symptoms (MCAS). The PeriMS index was computed as the sum of DRSP values at days 1, 2, 24, 25, 26, 27 and 28, and PeriMS was considered when the PeriMS index was $\geq$ 307 (0.666^{th} percentile of the distribution of the DRSP sums). The MCAS index was computed as sum of all DRSP scores from day 1 through day 28, and MCAS was considered when the index was $\geq$ 1050 (0.666^{th} percentile of the DRSP sum distribution).

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In our studies [11], we also examined the factor structure of the DRPS items in order to detect meaningful latent constructs that could be used as severity indices of relevant symptom domains. We detected 4 interpretable factors with eigenvalues > 1 and explaining 73.11% of the variance, with the first rotated principal factor (PC) explaining 20.14% of the variance and loading highly on depression, mood swings, sensitive to rejection, angry-irritable, more conflicts, less interest, out of control, and interference with hobbies and relationships, consequently named the *"depressive dimension"*. The second rotated PC explained 18.02% of the variance and loaded highly on concentration disturbances, lethargy, sleepiness, headache, muscle/joint pain and lowered productivity, named as the "*physio-somatic dimension*". The third rotated PC explained 17.83% of the variance and loaded highly on appetite and craving and breast tenderness and swelling, named the "*eating & breast PC*". The fourth rotated PC explained 17.11% of the variance and scored highly on hopelessness, anxious, lethargy, insomnia, being overwhelmed, and muscle-joint pain and was therefore named as "*anxiety PC*".

Clinical validation of the old and newly proposed PMS case definitions

In order to externally validate the different case definitions (i.e., PMS, ACOG, PeriMS, and MCAS) we examined the effects of diagnosis (differences in the DRSP score between women with and without the case definition) and time (4 time points) x diagnosis. The best case definition to capture increased severity of premenstrual symptoms was defined as the one that detects the strongest increase in the DRSP scores in the premenstrual period as indicated by the highest impact of the time x diagnosis interaction. The best case definition reflecting increased DRSP values all over the menstrual cycle is the one that showed the greatest differences in the sum of the 28 daily DRSP measurements between the 4 case definitions. Table 1 shows the

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Interestingly, we found that the PeriPMS and MCAS case definitions showed the most significant interaction patterns as well as the highest intergroup differences, while both the ACOG and PMS case definitions showed nearly no group differences and less significant interaction patterns. Thus, in women with the PeriMS and MCAS case definitions, higher DRSP values at all time points were found as compared to women without those case definitions while those differences were significantly pronounced in the week prior to the menses. As such, both the MCAS and PeriMS case definitions reflect increases in DRSP the week before the menses as well as higher DRSP levels during the whole menstrual cycle. Phrased differently, increased DRSP levels all over the cycle appear to be associated with highly significant increases in the DRSP score the week prior to the menses and a lowering of the scores the weeks after the menses.

In contrast, applying the ACOG diagnostic criteria showed that the DRSP score at T1, T2 and T3 were not significantly different between women with and without that case definition while the differences at T4 were only marginally different. As such, the diagnosis of ACOG does not reflect the actual degree of severity of symptoms in the premenstrual week. At least two flawed criteria of the ACOG diagnosis may explain that this case definition cannot be validated: (a) the criterion “reporting one or more affective or somatic symptoms during the 5 days prior to the menses” is very specific and too liberal; and (b) the criterion “symptoms should be relieved within 4 days after menses” may result in the omission of women with simultaneous increased premenstrual and postmenstrual scores. In fact, also the PMS case definition suffers from a similar flaw, namely the criterion “there should be a 30% difference in DRPS values between the premenstrual and postmenstrual week” will lead to exclusion of women with increased postmenstrual and premenstrual symptoms because the DRSP scores measured during the consecutive weeks are significantly intercorrelated during the menstrual cycle [12].

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Biomarkers validation of the PMS case definitions

We also examined the external validation of the four different case definitions using biomarkers of the menstrual cycle. Firstly, we examined whether sex hormones could be used to externally validate one or more of the case definitions. Therefore, we examined T1, T2, T3 and T4 estradiol and progesterone levels and found that the plasma steady-state levels of estradiol and progesterone were significantly lowered in subjects with PMS, PeriMS and MCAS, but not ACOG, as compared with women without that case definition [11]. Moreover, the diagnosis of PMS was only predicted by steady state levels of progesterone, while the PeriMS and MCAS diagnoses were significantly related to both estradiol and progesterone [11]. As such, the case definitions of PeriMS and MCAS, but not ACOG or PMS, were externally validated by lower levels of both sex hormones.

Secondly, we also examined changes in plasma levels of chemokines, namely CCL2 (C-C motif ligand 2), CCL5 (C-C motif ligand 5 or RANTES) and CCL11 (C-C motif ligand 11 or eotaxin), and EGF (epidermal growth factor) throughout the menstrual cycle in relation to the 4 case definitions [12]. This research revealed that CCL2, CCL5, CCL11 and EGF are significantly higher in women with MCAS than in women without MCAS, whereas there were no differences in those biomarkers between women with and without ACOG, PMS, and PeriMS. As such, the case definition MCAS was externally validated by increased plasma levels of chemokines and EGF, whereas PeriMS, PMS and ACOG could not be validated.

Thirdly, we also measured immune (e.g. complement C4) and oxidative stress (e.g. malondialdehyde (MDA), hydroperoxides (LOOH) and antioxidant enzymes, namely paraoxonase (PON)1 activity) biomarkers of affective disorders and their association with the 4 case definitions. We again observed significant associations between MCAS, but not ACOG, PMS and PeriMS, case definitions and lowered PON1 activity, increased LOOH and C4 levels

 doi:10.20944/preprints201912.0012.v1

(Roomruangwong et al., submitted). Nevertheless, in another biomarker study on IgA mediated immune responses to LPS of Gram-negative bacteria, there were no significant associations between the changes in the IgA responses to LPS and any of the 4 case definitions although changes in these biomarkers during the menstrual cycle were highly significantly correlated with changes in the DRSP score [13].

Biomarker validation of the total DRSP score and its 4 subdomains

Apart from externally validating different case definitions, it is also important to examine the changes in DRSP scores (total and subdomains) all over the menstrual cycle in association with changes in the biomarkers, and the mean DRSP score averaged over all time points in association with steady-state biomarker levels which are averaged over the menstrual cycle. Using repeated measurement design analyses, we found that the time series of the DRSP score was significantly and incersely associated with the time series in both progesterone and oestradiol levels and additionally with the steady state levels in progesterone. Similar patterns of prediction were established regarding severity of the *"depressive"* and *"physio-somatic"* dimensions. Moreover, we also examined distributed lag models whereby current as well as lagged (1 week) values of sex hormones predicted the changes in DRSP scores. Doing so, we observed that the *"depressive"* and *"physio-somatic"* symptom domain scores were significantly predicted by the lagged progesterone values, whilst the repeated measurements of the *"anxiety PC"* and the *"eating-breast PC"* were best predicted by steady state levels of progesterone and its lagged values [11].

Moreover, the DRSP and subdomain scores all over the menstrual cycle were significantly associated with steady-state levels of CCL2 and CCL5 (Roomruangwong et al., submitted) while a newly composed index reflecting the effects of the neurotoxic chemokines CCL2, CCL5 and CCL11 was significantly associated not only with the DRSP total score, but

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also with the *"depressive"*, *"physio-somatic"*, *"breast-craving"* and *"anxiety"* subdomain scores.

We also observed significant changes in the IgA levels directed to LPS of various gut commensal Gram-negative bacteria across the menstrual cycle with peak changes at T4 (day 28), but lows at T1 (day 7) and T2 (day 14). Additionally, changes in *Hafnia alvei, Morganella. morganii* and *Pseudomonas putida* were significantly associated with changes in the total DRSP scores and 2 subdomains namely *"physio-somatic"* and *"breast-craving"*. *H. alvei* was also detected to be associated with *"anxiety"* subdomain [13]. Lastly, we also found that changes in the DRSP score were significantly associated with changes in PON1 activity, MDA and C4 (lagged values) and that the severity of the subdomain scores was associated with those biomarkers or with LOOH levels (Roomruangwong et al., submitted).

Conclusions

Our results show that the discovery of new biomarkers of menstrual cycle-related symptoms is strongly dependent on the case definition used to assess those symptoms. The classical methods used to asses PMS including the ACOG case definition induce a high degree of type 2 errors or false-negative findings, while these methods do not allow to capture all aspects of the clinical picture including the continuous increase in DRSP scores and its subdomains during the menstrual cycle. Therefore, menstrual cycle-related symptoms cannot be adequately assessed using case definitions that are based on the severity of premenstrual symptoms coupled with a (partial) remission after the menses. In this paper we propose a new case definition of MCAS, which performed well and could be clinically and biologically validated. By inference, the best method to evaluate menstrual cycle-related symptoms and their associations with biomarkers is to assess daily measurements of those symptoms using the DRSP and based on those scores to a) make the diagnosis of MCAS, which reflects

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increased severity (overall and subdomains) during the menstrual cycle coupled with an incremental increase in the premenstrual period; b) examine the total DRSP score as well as its four subdomains; c) examine the associations of the time series in the DRSP score and those in biomarkers including using distributed lag models, which allow to predict the clinical scores by biomarkers measured some days earlier.

Authorships

CR and MM made the design of the study. Both authors agreed upon the final version of the paper.

Financial Support

This research has been supported by 1) the Ratchadaphiseksomphot Fund, Faculty of Medicine, Chulalongkorn University, grant number RA61/016; 2) Chulalongkorn University; Government Budget; and 3) the Ratchadaphiseksomphot Fund, Chulalongkorn University.

Conflict of interest

The authors have no conflict of interest with any commercial or other association in connection with the article.

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Table 1. Biomarker validation of four different case definitions of menstrual cycle-related symptoms

Clinical features and biomarker validation of the case definitions	**Case definitions**				**Associations with DRSP (total and subdomain) time series**
	PMS	**ACOG**	**PeriMS**	**MCAS**	
Interaction Time X case definition	mild↑	mild↑	↑↑	↑↑	NA
Differences in DRSP score	No	No	↑	↑↑	NA
Estradiol levels	No	No	↓↓	↓↓	↓
Progesterone levels	↓	No	↓↓	↓↓	↓↓
Chemokines (CCL2, CCL5, CCL11)	No	No	No	↑↑	↑↑
EGF	No	No	No	↑	-
IgA to LPS of Gram-negative bacteria	No	No	No	No	↑↑
PON1 activity	No	No	No	↓	↓
Hydroperoxides	No	No	No	↑	↑
Complement C4	No	No	No	↑	↑

DRSP: Daily Record of Severity of Problems (DRSP); NA: not applicable

PMS: Diagnosis of Premenstrual syndrome (PMS) by using DRSP cutoff value of 50 on the first day of menses with a DRSP score of ≥ 70 on day -5 to -1 of the menstrual cycle with at least 30% difference between the premenstrual and postmenstrual score.

 doi:10.20944/preprints201912.0012.v1

ACOG: Diagnosis of PMS according to the American College of Obstetricians and Gynecologists (ACOG) criteria which ≥ 1 affective and physical symptoms are present 5 days prior to the menses and remit within 4 days after the onset of menses without recurrence of symptoms until at least day 13 of the next cycle, for at least 3 consecutive menstrual cycles.

PeriMS: the diagnosis of Peri-Menstrual Syndrome using sum of DRSP values at days 1, 2, 24, 25, 26, 27, 28 ≥ 307 (0.666^{th} percentile of the distribution of the DRSP sums).

MCAS: The diagnosis of Menstrual-Cycle Associated Syndrome using sum of all DRSP scores from day 1 through day 28 ≥ 1,050 (0.666^{th} percentile of the DRSP sum distribution).

CCL2: C-C motif ligand 2, CCL5: C-C motif ligand 5 or RANTES, and CCL11: C-C motif ligand 11 or eotaxin

EGF: epidermal growth factor

IgA to LPS: IgA responses directed to LPS of Gram-negative bacteria

PON1: paraoxonase 1