Evaluation of serum antimullerian hormone and inhibin B concentrations in the differential diagnosis of secondary oligoamenorrhea

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Objective: To evaluate the performance of antimullerian hormone (AMH) and inhibin B as ovarian function markers for differentiating common causes of secondary oligoamenorrhea, namely hypogonadotrophic hypogonadism (HH), polycystic ovary syndrome (PCOS), premature ovarian failure (POF), and hyperprolactinemia (HPRL).

Design: Retrospective analysis.

Setting: Two university hospitals.

Patient(s): A total of 124 women with secondary oligoamenorrhea and 26 women with normal ovulation.

Intervention(s): Serum samples from the subjects were analyzed for AMH and inhibin B.

Main Outcome Measure(s): Serum AMH and inhibin B concentrations.

Result(s): Serum AMH concentration was significantly raised in women having World Health Organization group 2 anovulation, either with or without PCOS, and was significantly decreased to very low levels in POF; the diagnostic accuracy in both conditions was excellent, with areas under the receiver operating characteristic curve (AUC) of 0.913 and 0.977, respectively. The discriminatory performance between HH and PCOS was also good, with AUC 0.861. AMH remained unchanged in HH and HPRL compared with ovulatory control subjects. There were large overlap of serum inhibin B levels in the different conditions, and a significant difference from control subjects existed only in the POF group.

Conclusion(s): Serum AMH, but not inhibin B concentration, serves as a useful diagnostic tool in the differential diagnosis of secondary oligoamenorrhea. (Fertil Steril 2011;96:774–9. ©2011 by American Society for Reproductive Medicine.)

Key Words: Antimullerian hormone, inhibin B, hypogonadotrophic hypogonadism, polycystic ovary syndrome, premature ovarian failure, hyperprolactinemia

Secondary oligoamenorrhea is a common reproductive endocrine disorder among women in the reproductive age. The four most common causes of secondary oligoamenorrhea are: 1) hypogonadotrophic hypogonadism (HH); 2) normogonadotropic normogonadism; 3) hypergonadotropic hypogonadism (premature ovarian failure [POF]); and 4) hyperprolactinemia (HPRL) (1). The first three conditions were classified by the World Health Organization (WHO) as group 1, 2, and 3 anovulatory disorders, respectively. During the diagnostic evaluation of these patients, measurement of the hormonal profile, including serum FSH, E2, and PRL levels, usually helps in differentiating these conditions (2), aided by pelvic ultrasound scan for features of polycystic ovaries and determination of clinical and/or biochemical hyperandrogenism as part of the diagnostic criteria for PCOS (3).

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Antimullerian hormone (AMH) is a polypeptide of the transforming growth factor β family secreted solely by granulosa cells of the preantral and small antral ovarian follicles up to 9 mm (4), and its level in serum shows little fluctuation through the menstrual cycle (5, 6). Serum AMH level is considered to represent a reliable marker of the ovarian follicular pool and therefore could be a valuable diagnostic tool in ovulatory disorders. One study has investigated serum AMH levels in women with secondary amenorrhea (7). The AMH level in patients with functional hypothalamic amenorrhea (3.9 ± 1.5 ng/mL, i.e., 27.8 ± 10.7 pmol/L) was similar to normal control subjects (3.5 ± 1.5 ng/mL, i.e., 25.0 ± 10.4 pmol/L), whereas it is significantly higher in patients with PCOS (7.4 ± 1.7 ng/mL, i.e., 52.8 ± 12.1 pmol/L). In patients with POF, the AMH level is very low or undetectable (0–0.3 ng/mL, i.e., 0–2.1 pmol/L). Similar findings were also reported in other studies (8–10). Despite the small sample sizes, these reports confirmed a significant differentiation of the different types of ovulatory disorders by serum AMH measurement. Therefore, serum AMH could potentially serve as a useful tool in the diagnostic work-up of secondary oligoamenorrhoeic women.

Inhibin B is another hormone secreted from antral follicles, but in contrast to AMH, its production peaks at a follicular diameter of ~9–10 mm (4). Its role as an ovarian function test has also been explored. Serum inhibin B level has been variably reported to be lower (11) or normal (12) in women with HH. Serum inhibin B level was...
lower in young women with POF compared with ovulatory control subjects (13), and it declines with advancing age in women in the natural perimenopausal transition (14). Although some studies have reported an elevated level of inhibin B in women with PCOS, other have not confirmed this (15–19).

We conducted the present retrospective study to evaluate the performance of serum AMH and inhibin B measurements in the differential diagnosis of secondary oligoamenorrhea due to the four main causes of anovulatory disorder.

MATERIALS AND METHODS
Subjects
This was a retrospective analysis using stored serum samples. Archived serum samples from a total of 102 patients with secondary oligoamenorrhea (with cycle length >35 days) attending the Department of Obstetrics and Gynaecology, Queen Mary Hospital, Hong Kong, and the Edinburgh Fertility and Reproductive Endocrine Centre, Royal Infirmary of Edinburgh, United Kingdom, were retrieved. Another 26 serum samples from normally ovulating women with regular cycles were used as control samples. Samples were collected with informed consent. Ethics approval of the study was obtained from the Institutional Review Board, University of Hong Kong/Hospital Authority Hong Kong West Cluster. Serum samples were assayed for AMH and inhibin B concentration. The archival serum samples had been stored at −20 °C for up to 3 years before the present analysis. It has been reported that AMH immunoreactivity was stable through sample storage at room temperature for several days and through multiple cycles of freezing and thawing (20).

The diagnostic groups of the studied subjects are described in Table 1. Subjects in the PCOS group were diagnosed by two out of the three criteria according to the Rotterdam consensus (3). Subjects with other coexisting identifiable causes of secondary amenorrhea, e.g., drug-induced condition, abnormal thyroid dysfunction, or within 6 weeks of a recent pregnancy, were excluded from study. None of the subjects had breastfed within the 6 months preceding the blood test. Another 22 subjects with normogonadotropic normogonadic (WHO group 2) anovulation who did not fulfill the Rotterdam criteria for PCOS were also studied.

Hormonal Tests
The test blood samples were taken in the early follicular phase (day 2–4) of a spontaneous period in women with regular periods or a progestogen-induced withdrawal bleed in women with long/irregular cycles. AMH concentration was determined using a second generation enzyme immunoassay kit (ImmunoTech; Beckman-Coulter; reference A16507), which has a sensitivity of 0.7 pmol/L. The inter- and intra-assay coefficients of variation were <14.2% and <12.3%, respectively. Inhibin B concentration was determined by a two-site enzyme-linked immunoassay (Serotec; Kidlington). That assay sensitivity is 15.6 pg/mL, and the inter- and intra-assay coefficients of variation were <7%.

Statistics
Because the AMH and inhibin B levels of our subjects were not normally distributed, the values in the different groups were compared with the use of the Kruskal-Wallis test with Dunn post hoc analysis. The predictive value of serum AMH and inhibin B levels on the differential diagnosis of secondary amenorrhea was analyzed with the use of the receiver operating characteristic (ROC) curve. Statistical analyses were performed using GraphPad Prism 4.0 software.

For comparison of serum AMH levels in different groups of amenorrhoic women and normal controls, assuming that a difference of 1 SD in the serum AMH level among the groups was clinically relevant, a sample size of 22 per group was adequate to determine a statistical significance at power of 90% and type 1 error of 0.05.

RESULTS
The median (range) of the age and endocrinologic parameters of the subject groups are presented in Table 1.

Subjects with HH had significantly lower FSH, LH, and E2 concentrations compared with control subjects, whereas those with POF had significantly higher FSH and LH but lower E2 concentrations compared with control subjects. The FSH, LH, and E2 concentrations of subjects with PCOS and HPRL were not different from the control subjects. These are compatible with the WHO definition for the respective groups of anovulatory women (1, 2).

All but three of the subjects in the POF group were amenorrhoic. For the three who were oligomenorrhoic, their hormone profile, including AMH concentration, did not differ significantly from the rest of the group who were amenorrhoic.

The median AMH concentration in subjects with PCOS was significantly higher (P<.001) compared with control subjects, whereas in those with POF it was significantly lower (P<.001). The median serum AMH concentration in subjects with HH and HPRL were not significantly different from that of ovulatory control subjects (P>.05) (Table 1; Fig. 1A).

The median serum inhibin B concentration in subjects with HH, PCOS, and HPRL were not significantly different from that of ovulatory controls (P>.05), but the median level in those with POF was significantly lower (P<.001) than in control subjects (Table 2; Fig. 1B).

### TABLE 1

<table>
<thead>
<tr>
<th>Subject group</th>
<th>Description</th>
<th>Diagnostic criteria</th>
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<tbody>
<tr>
<td>Control</td>
<td>Normal ovulatory control subjects</td>
<td>Regular menstruation with documented ovulatory cycles</td>
</tr>
<tr>
<td>WHO 1 (HH)</td>
<td>Hypogonadotropic hypogonadism</td>
<td>Secondary oligoamenorrhea with normal or low serum FSH and low E2 levels</td>
</tr>
<tr>
<td>WHO 2 (including PCOS and non-PCOS)</td>
<td>Normogonadotropic normogonadic anovulation</td>
<td>Secondary oligoamenorrhea with normal serum FSH and E2 levels (PCOS was diagnosed with Rotterdam criteria)</td>
</tr>
<tr>
<td>WHO 3 (POF)</td>
<td>Hypergonadotropic hypogonadism</td>
<td>Secondary oligoamenorrhea with raised FSH levels (&gt;20 IU/L) over two occasions ≥ 6 weeks apart</td>
</tr>
<tr>
<td>HPRL</td>
<td>Hyperprolactinemia</td>
<td>Secondary oligoamenorrhea with raised PRL level (&gt;550 mIU/L)</td>
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</table>

Note: HH = hypogonadotropic hypogonadism; HPRL = hyperprolactinemia; PCOS = polycystic ovary syndrome; POF = premature ovarian failure.

The serum AMH concentration was further analyzed after adding subjects with WHO group 2 anovulation who did not fulfill the Rotterdam criteria for PCOS [WHO 2 (non-PCOS)]. The combined group comprising both PCOS and WHO 2 (non-PCOS) subjects [WHO 2 (all)] still had significantly higher serum AMH compared with control subjects (P < 0.01) as well as subjects with HH (P < 0.01), POF (P < 0.01), and HPRL (P < 0.01; Fig. 1A). Serum AMH was significantly higher in both the PCOS (P < 0.01) and the WHO 2 (non-PCOS) (P < 0.05) groups compared with control subjects (Fig. 1C).

The ROC curves of serum AMH concentration in discriminating PCOS, WHO 2 (all), and POF from control subjects, as well as in distinguishing between HH and PCOS, are depicted in Figure 2; the area under the curves (AUC) were 0.913 (95% confidence interval [CI] 0.843–0.982), 0.849 (0.766–0.932), 0.977 (95% CI 0.944–1.009), and 0.861 (95% CI 0.767–0.955), respectively. Using a cutoff AMH concentration at 42 pmol/L gave an optimal sensitivity of 79% and specificity of 96% in differentiating PCOS from control subjects, and at 8 pmol/L it gave an optimal sensitivity of 85% and specificity of 100% in diagnosing POF.

DISCUSSION

The present study confirmed the findings in earlier reports (7, 13, 21) that serum AMH levels are significantly higher in women with PCOS and significantly lower in women with POF, whereas they remain unchanged in HH. We also demonstrate for the first time that serum AMH remains unchanged in HPRL despite the associated hypogonadotropism. Moreover, to our knowledge, this is also the first report incorporating WHO 2 subjects who did not fulfill the full Rotterdam criteria for PCOS (3), who are not uncommonly encountered in clinical practice. Our results confirm that serum AMH was also increased in WHO 2 (non-PCOS) compared with control subjects, although to a lesser extent than in PCOS, and it was significantly lower than in PCOS.

Regarding serum inhibin B, its value in diagnosing the various causes of amenorrhea was more limited than earlier reports might suggest. There are reports that inhibin B was decreased (11) or normal (12) in HH. In PCOS, there are reports of serum inhibin B being elevated or unchanged compared with control subjects (15–19). It has been reported that serum inhibin B levels are lower in women with POF (13). However, a recent study revealed that they were higher in POF due to autoimmune oophoritis but decreased in idioopathic POF, thus potentially discriminating between the two conditions (22). A normal inhibin B level was also revealed in “resistant ovarian syndrome” in a case report (23), although formal comparative study is lacking. Our results showed a significant difference in serum inhibin B compared with control subjects only in the POF group but not in HH, PCOS, or HPRL. Therefore, it does not have

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### TABLE 2
Age and endocrinological parameters of study subjects, median (interquartile range).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HH</th>
<th>PCOS</th>
<th>POF</th>
<th>HPRL</th>
</tr>
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<tbody>
<tr>
<td><strong>n</strong></td>
<td>26</td>
<td>24</td>
<td>33</td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td>Age (y)</td>
<td>34.9 (32.5–36.8)</td>
<td>25.5 (18.0–34.0)</td>
<td>27.5 (24.8–31.0)</td>
<td>33.0 (31.0–36.0)</td>
<td>34.5 (28.8–40.3)</td>
</tr>
<tr>
<td>FSH (IU/L)</td>
<td>6.1 (3.1–8.8)</td>
<td>2.1 (0.4–4.3)</td>
<td>5.7 (4.3–67.0)</td>
<td>87.4 (70.7–136.6)</td>
<td>6.6 (5.2–8.4)</td>
</tr>
<tr>
<td>LH (IU/L)</td>
<td>6.2 (4.4–8.4)</td>
<td>0.3 (0.1–1.3)</td>
<td>11.2 (7.2–15.5)</td>
<td>30.4 (25.2–54.8)</td>
<td>4.4 (1.9–7.3)</td>
</tr>
<tr>
<td>E2 (pmol/L)</td>
<td>222 (127–425)</td>
<td>38 (21–69)</td>
<td>146 (122–202)</td>
<td>44 (24–71)</td>
<td>129 (63–268)</td>
</tr>
<tr>
<td>AMH (pmol/L)</td>
<td>20.6 (12.1–33.5)</td>
<td>23.2 (10.5–32.6)</td>
<td>65.7 (47.2–92.8)</td>
<td>2.5 (1.1–3.7)</td>
<td>18.0 (13.3–29.5)</td>
</tr>
<tr>
<td>Inhibin B (pg/mL)</td>
<td>41.4 (&lt;15.6–69.0)</td>
<td>10.9 (&lt;15.6–22.0)</td>
<td>64.1 (33.5–97.4)</td>
<td>&lt;15.6 (&lt;15.6–15.6)</td>
<td>47.0 (&lt;15.6–94.7)</td>
</tr>
</tbody>
</table>

**Note:** AMH = antimullerian hormone; other abbreviations as in Table 1.

\(^a P < .001\) compared with control.

\(^b P < .05\) compared with control.


### FIGURE 2
Receiver operating characteristic (ROC) curves of serum AMH in discrimination of (A) polycystic ovary syndrome (PCOS) versus control; (B) World Health Organization group 2 anovulation (all) versus control; (C) ovarian failure versus control; and (D) PCOS versus hypogonadotropic hypogonadism.

good discriminatory performance and is not useful in clinical diagnosis.

We evaluated the diagnostic performance of serum AMH with the use of ROC analysis. The AUCs of 0.913 and 0.977 for diagnosing PCOS and POF, respectively, versus control subjects indicates an excellent performance of serum AMH in diagnosing these two conditions. The diagnostic performance of AMH on PCOS in our study was similar to, if not better than previously reported (21). When applied to the whole group of WHO 2 (all), serum AMH still had an AUC of 0.849, although slightly dampened by the WHO 2 (non-PCOS) subjects because this group had serum AMH levels lying intermediate between PCOS and control subjects. Our findings showed the same trend as previously reported (24).

When used in clinical diagnosis, it is useful to set a cutoff value to achieve optimal specificity with acceptable sensitivity. Our data suggest a cutoff AMH concentration at 42 pmol/L in diagnosing PCOS (sensitivity 79%, specificity 96%), and at 8 pmol/L in diagnosing POF (sensitivity 85%, specificity 100%). These data suggest an excellent potential for serum AMH assay to be used clinically for the differential diagnosis of anovulatory conditions in replacement of conventional ovarian function markers, such as serum FSH and E2. Although the latter have been established as the standard diagnostic tests according to the WHO classification, they vary with the menstrual cycle and therefore the blood test needs to be timed to the early follicular phase for meaningful interpretation, except in women who are completely amenorrheic and therefore consistently anovulatory. This often causes inconvenience to the patient and the clinic logistically. AMH is also not operator dependent, unlike ultrasound ovarian morphology. Moreover, recent work from our group and others also showed that serum AMH level was not affected by the use of exogenous hormones, e.g., combined hormonal contraceptives and progestogen-only contraceptives (25, 26), which the patients might have been taking before referral for specialist consultation. Furthermore, there is often overlap between HH and PCOS subjects based on serum FSH, and some patients with HH are only marginally hypogonadic. Our results suggest that in addition to FSH and E2 concentrations, serum AMH may contribute further information for differentiating HH from PCOS, although it may require larger studies to confirm the delineation.

One major limitation of applying serum AMH assay as a clinical test is the lack of an international standard. Currently, there are two commercial ELISA kits for the purpose, which produce discrepant though well correlated results (27). With more data from larger scale studies in different populations, standardized criteria for diagnosis will hopefully be derived in the future. An automated system for AMH assay is also awaited, which would facilitate more efficient and widespread application as a clinical test.

In summary, the present study confirmed that serum AMH concentration is raised in women with WHO group 2 anovulatory disorder, either with or without PCOS, and is decreased to very low levels in POF; the diagnostic accuracy in both conditions was excellent. AMH was unchanged in HH and HPRL compared with ovulatory control subjects. Serum AMH also had good discriminatory performance between HH and PCOS. In contrast, serum inhibin B has large overlaps in the different conditions. Therefore, serum AMH, but not inhibin B concentration, can serve as a useful diagnostic tool in the differential diagnosis of secondary amenorrhoea.

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