

# A decision-making algorithm for performing or cancelling embryo transfer in patients at high risk for ovarian hyperstimulation syndrome after triggering final oocyte maturation with hCG

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Submitted on September 25, 2019; resubmitted on January 23, 2020; editorial decision on February 11, 2020

**STUDY QUESTION:** Can the grade of ascites, haematocrit (Ht), white blood cell (WBC) count and maximal ovarian diameter (MOD) measured on Day 3 be used to construct a decision-making algorithm for performing or cancelling embryo transfer in patients at high risk for severe ovarian hyperstimulation syndrome (OHSS) after an hCG trigger?

**SUMMARY ANSWER:** Using cut-offs of ascites grade >2, Ht >39.2%, WBC >12 900/mm<sup>3</sup> and MOD >85 mm on Day 3, a decision-making algorithm was constructed that could predict subsequent development of severe OHSS on Day 5 with an AUC of 0.93, a sensitivity of 88.5% and a specificity of 84.2% in high-risk patients triggered with hCG.

**WHAT IS KNOWN ALREADY:** Despite the increasing popularity of GnRH agonist trigger for final oocyte maturation as a way to prevent OHSS, ≥75% of IVF cycles still involve an hCG trigger. Numerous risk factors and predictive models of OHSS have been proposed, but the measurement of these early predictors is restricted either prior to or during the controlled ovarian stimulation. In high-risk patients triggered with hCG, the identification of luteal-phase predictors assessed post-oocyte retrieval, which reflect the pathophysiological changes leading to severe early OHSS, is currently lacking.

**STUDY DESIGN, SIZE, DURATION:** A retrospective study of 321 patients at high risk for severe OHSS following hCG triggering of final oocyte maturation. High risk for OHSS was defined as the presence of at least 19 follicles ≥11 mm on the day of triggering of final oocyte maturation.

**PARTICIPANTS/MATERIALS, SETTING, METHODS:** The study includes IVF/ICSI patients at high risk for developing severe OHSS, who administered hCG to trigger final oocyte maturation. Ascites grade, MOD, Ht and WBC were assessed in the luteal phase starting from the day of oocyte retrieval. Outcome measures were the optimal thresholds of ascites grade, MOD, Ht and WBC measured on Day 3 post-oocyte retrieval to predict subsequent severe OHSS development on Day 5. These criteria were used to construct a decision-making algorithm for embryo transfer, based on the estimated probability of severe OHSS development on Day 5.

**MAIN RESULTS AND THE ROLE OF CHANCE:** The optimal Day 3 cutoffs for severe OHSS prediction on Day 5 were ascites grade >2, Ht >39.2%, WBC >12 900/mm<sup>3</sup> and MOD >85 mm. The probability of severe OHSS with no criteria fulfilled on Day 3 is 0% (95% CI: 0–5.5); with one criterion, 0.8% (95% CI: 0.15–4.6); with two criteria, 13.3% (95% CI: 7.4–22.8); with three criteria, 37.2% (95% CI: 24.4–52.1); and with four criteria, 88.9% (95% CI, 67.2–98.1). The predictive model of severe OHSS had an AUC of 0.93 with a sensitivity of 88.5% and a specificity of 84.2%.

**LIMITATIONS, REASONS FOR CAUTION:** This is a retrospective study, and therefore, it cannot be excluded that non-apparent sources of bias might be present. In addition, we acknowledge the lack of external validation of our model. We have created a web-based calculator (<http://ohsspredict.org>), for wider access and usage of our tool. By inserting the values of ascites grade, MOD, Ht and WBC of high-risk patients on Day 3 after oocyte retrieval, the clinician instantly receives the predicted probability of severe OHSS development on Day 5.

**WIDER IMPLICATIONS OF THE FINDINGS:** The present study describes a novel decision-making algorithm for embryo transfer based on ascites, Ht, WBC and MOD measurements on Day 3. The algorithm may be useful for the management of high-risk patients triggered with hCG and for helping the clinician's decision to proceed with, or to cancel, embryo transfer. It must be emphasized that the availability of the present decision-making algorithm should in no way encourage the use of hCG trigger in patients at high risk for OHSS. In these patients, the recommended approach is the use of GnRH antagonist protocols, GnRH agonist trigger and elective embryo cryopreservation. In addition, in patients triggered with hCG, freezing all embryos and luteal-phase GnRH antagonist administration should be considered for the outpatient management of severe early OHSS and prevention of late OHSS.

**STUDY FUNDING/COMPETING INTEREST(S):** NHMRC Early Career Fellowship (GNT1147154) to C.A.V. No conflict of interest to declare.

**TRIAL REGISTRATION NUMBER:** N/A.

**Key words:** ovarian hyperstimulation syndrome / prediction / decision making / embryo transfer / high risk / ascites / haematocrit / white blood cell count / maximal ovarian diameter

## WHAT DOES THIS MEAN FOR PATIENTS?

Ovarian hyperstimulation syndrome (OHSS) is a serious and potentially life-threatening complication of ovarian stimulation that affects up to 2 out of 100 women having IVF/ICSI treatment. It can be mild, moderate or severe. The severe form of OHSS usually requires long hospital treatment, with a considerable cost for both the patient and national health systems. OHSS causes deaths of mothers, and three deaths per 100 000 women undergoing ovarian stimulation have been reported worldwide.

According to ESHRE, 2040 cases of OHSS were reported from European countries for the year 2014. More importantly, it seems that OHSS is not always reported by clinics, since there is a big difference between the number of cases reported to the UK regulator Human Fertilisation and Embryology Authority (HFEA) and the number of hospital admissions due to OHSS reported to NHS Digital. Sixty cases of serious or critical OHSS were reported to the HFEA in 2015 and 38 cases in 2016 compared with the actual 865 admissions to hospital for OHSS in England during the same period, 836 of which were emergency admissions.

The authors therefore want OHSS to be more accurately predicted so it can be prevented.

In this paper, the authors develop a decision-making tool for doctors that predict severe OHSS in women undergoing IVF/ICSI treatment. This tool is very accurate at predicting severe OHSS and can do so early in the IVF/ICSI process.

The tool is useful for telling doctors when severe OHSS is likely to happen to women who are having IVF. This would then help them give these women advice early on in their treatment and therefore allow decisions about treatment to be made at an early stage.

## Introduction

Ovarian hyperstimulation syndrome (OHSS) is a serious and potentially life-threatening iatrogenic complication of ovarian stimulation, triggered by hCG (Rizk et al., 1997). OHSS is associated with maternal mortality, with a reported frequency of three deaths per 100 000 women undergoing ovarian stimulation (Confidential Enquiry into Maternal and Child Health, 2007; Braat et al., 2010). Therefore, it becomes apparent that OHSS is a complication that needs to be predicted, diagnosed and eliminated effectively.

Despite the increasing popularity of GnRH agonist trigger for final oocyte maturation as a measure to make OHSS a complication of the past in women at high risk for OHSS (OHSS-free clinic concept) (Devroey et al., 2011; Donoso and Devroey, 2013), hCG administration remains the most popular triggering option, with  $\geq 75\%$  of IVF cycles worldwide involving the use of hCG trigger (De Ziegler and Shoham, 2013; Tobler et al., 2014).

According to the European IVF-monitoring Consortium of ESHRE, 2040 cases of OHSS were reported from European countries for the

year 2014 (De Geyter et al., 2018). More importantly, it seems that OHSS is under-reported by clinics, as shown by the wide disparity between the number of cases reported to the HFEA and the number of hospital admissions due to OHSS reported to NHS Digital. Sixty cases of serious or critical OHSS were reported to the HFEA in 2015 and 38 cases in 2016 compared with the 865 admissions to hospital for OHSS in England during the same period, 836 of which were emergency admissions (HFEA, 2018).

A significant contributing factor for the popularity of hCG trigger, even in high-risk patients, is also the fact that the long GnRH agonist protocol, which is incompatible with agonist triggering, has the highest usage among clinics compared with antagonist protocols (Tur-Kaspa and Fauser). As a result, OHSS still occurs on a worldwide scale (Calhaz-Jorge et al., 2017). The incidence of severe OHSS among high-risk patients ranges from 9% to 38% (Asch et al., 1991; Morris et al., 1995; Swanton et al., 2010; Lainas et al., 2012).

Numerous OHSS risk factors and predictive models have been proposed (Enskog et al., 1999; Practice Committee of the American Society for Reproductive Medicine, 2008; Humaidan et al., 2010; Leitao

*et al.*, 2014; Nastri *et al.*, 2015; Bechtejew *et al.*, 2017). However, the measurement of these early predictors is restricted to either prior to or during the controlled ovarian stimulation. In patients triggered with hCG, the identification of luteal-phase predictors assessed post-oocyte retrieval, which reflect the pathophysiological changes leading to severe early OHSS (Lainas *et al.*, 2018), is currently lacking. Such predictors would facilitate the prediction and diagnosis of early OHSS and inform preventative clinical interventions, such as embryo transfer cancellation and embryo cryopreservation.

The aim of the present study was to identify luteal-phase parameters assessed 3 days post-oocyte retrieval in order to construct a decision-making algorithm for performing or cancelling embryo transfer in patients at high risk for severe OHSS triggered with hCG.

## Materials and Methods

### Study design and patient population

This is a retrospective cohort study including 321 patients at high risk for severe OHSS following hCG triggering of final oocyte maturation (Fig. 1).

All patients were previously included in a study describing the ultrasound and haematological changes during the early luteal phase in the presence of high risk for developing OHSS (Lainas *et al.*, 2018).

The patients underwent IVF treatment from January 2009 until December 2012 at Eugonia ART Unit in Athens. The present study included patients at high risk for severe OHSS, defined as the presence of at least 19 follicles  $\geq 11$  mm on the day of triggering of final oocyte maturation with hCG (Griesinger *et al.*, 2016). Patients were included in the analysis only once.

Patients receiving GnRH agonist trigger or withholding hCG, as well as those with incomplete data, were excluded.

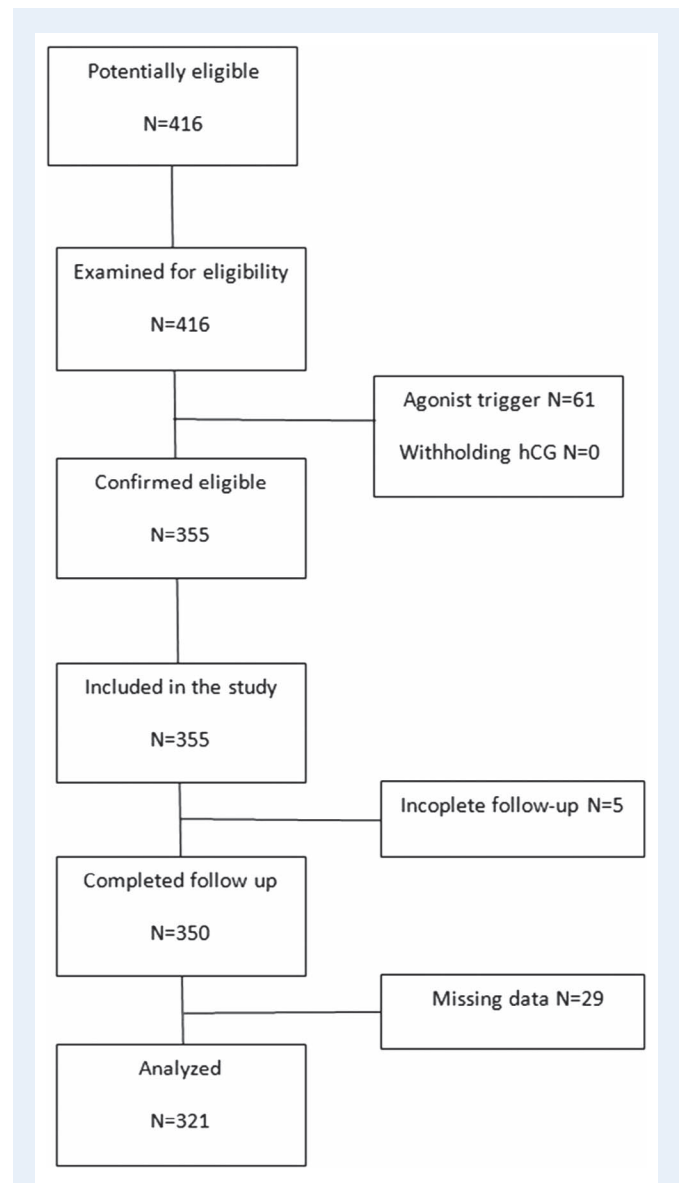
Patients reaching the final analysis are shown in Fig. 1, which includes the numbers included, excluded, loss of follow-up and data loss.

The present study received ethics approval from the Institutional Review Board of Eugonia (approval number, 02/28-09-13).

### Ovarian stimulation and triggering of final oocyte maturation

Patients underwent ovarian stimulation for IVF/ICSI using either a long GnRH agonist protocol or a flexible GnRH antagonist protocol, as previously described (Lainas *et al.*, 2010).

Ovarian stimulation was performed with a starting dose of 150 IU/day of recombinant FSH (rFSH, Gonal-F; Merck Serono, Geneva, Switzerland). This dose was adjusted after Day 5 of stimulation, depending on the ovarian response, as assessed by oestradiol (E2) levels and ultrasound. When  $\geq 3$  follicles of a mean diameter  $\geq 17$  mm were present, the following options were offered to all high-risk for severe OHSS patients: withholding hCG and cancellation of the cycle; triggering of final oocyte maturation using GnRH agonist instead of hCG and cryopreservation of all embryos, if patients had been treated with a GnRH antagonist protocol; or proceeding to oocyte retrieval using low 5000 IU hCG for triggering final oocyte maturation (Kolibianakis *et al.*, 2007) followed by embryo transfer, if severe OHSS did not develop by Day 5 post-oocyte retrieval or by luteal GnRH antagonist administration and embryo cryopreservation,



**Figure 1** Flow chart for the selection of study participants at high risk for ovarian hyperstimulation syndrome after triggering final oocyte maturation with hCG.

if severe OHSS developed by Day 5 post-oocyte retrieval (Lainas *et al.*, 2012).

Patients who were eligible for inclusion in the current study opted for triggering final oocyte maturation with hCG, since they wished to proceed at least to oocyte retrieval and retain the possibility of a fresh embryo transfer in case severe OHSS did not develop.

### Oocyte retrieval, fertilization and embryo culture

Transvaginal ultrasound-guided oocyte retrieval was performed by double lumen needle aspiration 36 hours following hCG administration. ICSI was performed only in cases with severe male factor or previous fertilization failure. Embryos were cultured in sequential media (Origio, Malov, Denmark) for 5–6 days to the blastocyst stage.

**Table 1** Classification of ascites.

Grade 1	No ascites	Absence of fluid
Grade 2	Low	Small amount of fluid, barely detectable by ultrasound in the pouch of Douglas
Grade 3	Moderate	Increased amount of fluid located in the small pelvis
Grade 4	Marked	Large amount of fluid reaching the level of the umbilicus
Grade 5	Massive	Significant accumulation of fluid reaching Morrison's pouch
Grade 6	Tense	Significant accumulation of fluid up to the level of the diaphragm with/without hydrothorax

## Criteria for the diagnosis of severe OHSS

The criteria used for the diagnosis of severe OHSS are a modification of the classification initially proposed by Navot et al. (1992). Severe OHSS was diagnosed in the presence of at least moderate (Grade 3) ascites and two or more of the following: enlarged ovaries ( $\geq 100$  mm maximal ovarian diameter (MOD)), haematocrit (Ht)  $\geq 45\%$ , white blood cell (WBC) count  $\geq 15\,000/\text{mm}^3$ , hydrothorax, dyspnea or oliguria, as previously described (Lainas et al., 2012, 2013, 2018).

Ascites was classified in six grades (Table 1) depending on the quantity of fluid accumulation in relation to anatomical regions in the peritoneal cavity, as previously described (Lainas et al., 2012, 2013, 2014).

## Management of severe early OHSS

Patients diagnosed with severe early OHSS were managed on an outpatient basis by the administration of GnRH antagonist and cryopreservation of all blastocysts, as previously described (Lainas et al., 2009; Lainas et al., 2012, 2014). GnRH antagonist was administered for 3–5 days starting on the day of severe OHSS diagnosis.

## Patient follow-up

Monitoring of all patients for the development of severe early OHSS was performed on the day of oocyte retrieval (Day 0), 3 days (Day 3) and 5 days post-oocyte retrieval (Day 5), which is the standard policy of the center for all high-risk patients. Patient follow-up was continued on Days 7, 9 and 11 post-oocyte retrieval to ensure complete resolution of early OHSS and to detect possible signs of late OHSS development, as previously reported (Lainas et al., 2012). The present study comprises the first 5 days post-oocyte retrieval (from Day 0 until Day 5).

## Ultrasound and laboratory assays

Ultrasound assessment of ascites was performed on the day of oocyte retrieval (Day 0), 3 days (Day 3) and 5 days post-oocyte retrieval (Day 5) and every 2 days thereafter up to Day 11 post-oocyte retrieval.

Ovarian size was assessed by transvaginal ultrasound scan 3 and 5 days post-oocyte retrieval and every 2 days thereafter up to Day 11 post-oocyte retrieval. The operator scanned the ovaries in at

least three different planes and identified the plane with the maximal diameter for each ovary. These two diameters were compared and only the largest diameter of the two ovaries was registered as MOD.

Ultrasound measurements of ascitic fluid and ovarian diameter were performed using a 7.5 or 6 or 5 MHz vaginal probe (Sonoline Adara, Siemens).

Measurements of Ht and WBC were performed on the day of oocyte retrieval (Day 0), 3 days (Day 3) and 5 days post-oocyte retrieval (Day 5) and every 3 days thereafter up to Day 11 post-oocyte retrieval. Ht and WBC were determined by flow cytometry using Coulter A<sup>c</sup>.Tdiff™ Analyzer (Coulter Corporation, Miami, FL, USA). The coefficient of variation, specifying imprecision limits for WBC and red blood cell count, was 3%.

## Outcome measures

Outcome measures were the optimal thresholds (with their sensitivity and specificity) of the absolute numbers and changes of Ht, ascites grade, WBC and MOD, measured 3 days after oocyte retrieval to predict subsequent severe OHSS development. An additional objective was the construction of a decision-making model for the prediction of severe early OHSS based on Day 3 measurements.

## Statistical analysis

The independent variables are the ultrasound assessment of ascites grade and MOD and haematological measurements of Ht and WBC on Day 0 (day of oocyte retrieval) and Day 3 post-oocyte retrieval.

The measurements of the variables assessed, as well as their differences between Days 0 and 3, were subjected to receiver operating characteristic (ROC) curve analysis to evaluate whether they possess significant discrimination ability for the development of severe early OHSS. In each case, the AUC with its 95% CI was reported. This was followed by the identification for each variable of the best cut-off value that yields the optimal trade-off between sensitivity and specificity for the prediction of OHSS: Youden index (Youden, 1950).

For the construction of the predictive model, the dichotomous Day 3 parameters produced at the previous step were tested for their predictive ability on OHSS, first with univariate statistics using of  $2 \times 2$  contingency tables, calculating the odds ratios (ORs) with their 95% CI. Subsequently, the independent predictors were subjected to multivariate analysis with the use of the stepwise logistic regression approach, once again reporting the relevant OR with their 95% CI.

The results of the logistic regression equation were used to build the final prediction model and define its discrimination ability for predicting severe early OHSS.

## Results

A flow diagram reporting patient numbers at each stage of the study is shown in Fig. 1. Severe early OHSS was diagnosed in 43 out of 321 (13.4%; 95% CI: 10.2–17.7) women on Day 5 post-oocyte retrieval.

A total of 278 women not diagnosed with severe OHSS proceeded to blastocyst transfer, resulting in 213 positive hCG tests (76.6%; 95% CI: 71.3–81.2), 181 clinical pregnancies (65.1%; 95% CI: 59.3–70.5),

**Table II** Baseline characteristics, ovarian stimulation and embryological data of women with and without severe early OHSS.

	Severe early OHSS		P-value
	No (278)	Yes (43)	
Age, mean (SD), years	32.5 (4.4)	32.5 (4.5)	0.953
BMI, mean (SD), kg/m <sup>2</sup>	23.5 (4.0)	24.4 (4.0)	0.158
Duration of infertility, median (IQR), years	3 (2)	3 (3)	0.502
Previous attempts, median (IQR)	0 (2)	0 (1)	0.610
Dose of gonadotrophins, median (IQR), IU	1800 (890)	1500 (850)	0.033
AFC, median (IQR)	21 (6)	22 (6)	0.294
Basal LH, median (IQR), IU/l	5.8 (2.2)	5.6 (3.1)	0.436
Basal FSH, median (IQR), IU/l	7.2 (2.2)	5.8 (2.5)	0.001
Basal oestradiol, median (IQR), pg/ml	30 (18)	32 (15)	0.944
Basal progesterone, median (IQR), ng/ml	0.47 (0.37)	0.43 (0.36)	0.318
Oestradiol on hCG day, median (IQR) (pg/ml)	2787 (1639)	3806 (2255)	0.001
Progesterone on hCG day, median (IQR) (ng/ml)	0.95 (0.55)	0.97 (0.62)	0.470
Days of stimulation, median (IQR), days	11 (2)	10 (1)	0.142
Number of follicles on hCG day, median (IQR)	29 (5)	29 (10)	0.677
Number of oocytes retrieved, median (IQR)	23 (8)	27 (13)	0.001
Number of 2PN	14 (7)	16 (9)	0.003
Agonist protocol, n (%)	158 (56.8%)	14 (32.6%)	0.005
Antagonist protocol	120 (43.2%)	29 (67.4%)	

OHSS indicates ovarian hyperstimulation syndrome; IQR, interquartile range; AFC, antral follicle count; 2PN, two pronuclei.

Variables that are normally distributed are expressed as mean (SD) and were analysed with independent sample t-test. Variables that are not normally distributed are expressed as median (IQR) and were analysed with Mann–Whitney test.

158 ongoing pregnancies (56.8%; 95% CI: 50.1–62.5) and 136 live births (48.9%; 95% CI: 43.1–54.8).

No patient developed severe early OHSS prior to Day 5. None of the women with a positive pregnancy test developed late-onset OHSS.

The characteristics of high-risk patients included in the analysis are shown in Table II. The results of the ROC analysis of all parameters are shown in Table III.

### Parameters on the day of oocyte retrieval (Day 0)

All Day 0 variables, except ascites grade, had significant discrimination ability on subsequent OHSS development, the most significant being the number of oocytes retrieved, with 0.70 AUC (95% CI: 0.62–0.78) (Table III). For the prediction of severe early OHSS, the optimal cutoff point (Youden index) for the number of oocytes was >25, with 67.4% sensitivity and 62.7% specificity. This yields an OR = 3.5 (95% CI 1.8–7.0).

### Parameters on Day 3 post-oocyte retrieval

By performing an ROC curve analysis, the best single predictor for development of severe OHSS was ascites grade on Day 3 (AUC: 0.88; 95% CI: 0.84–0.92), which was significantly better ( $P = 0.004$ ) than the next optimal predictor, which was WBC on Day 3 (AUC: 0.75; 95%

CI: 0.70–0.80; difference between AUCs: 0.13; 95% CI: 0.04–0.22) (Table III).

The optimal Day 3 cutoffs for severe OHSS prediction were as follows: ascites grade >2, Ht >39.2%, WBC >12 900/mm<sup>3</sup> and MOD >85 mm.

### Changes between Day 3 and Day 0

By performing an ROC curve analysis, the best single predictor for the development of severe OHSS was cAsc<sub>D3-D0</sub> (AUC: 0.88; 95% CI: 0.83–0.91), which was significantly better ( $P = 0.001$ ) than the next optimal predictor, which was cWBC<sub>D3-D0</sub> (AUC: 0.68; 95% CI: 0.62–0.73; difference between AUCs: 0.20; 95% CI: 0.09–0.30) (Table III).

### Construction of a prediction model for the occurrence of severe early OHSS

A stepwise logistic regression analysis with OHSS occurrence as the dependent variable and all the significant cut-off parameters calculated in Table III as the independent predictors was performed. The variables that entered the logistic regression equation were the four Day-3 variables: ascites grade (>2), Ht (>39.2%), WBC (>12 900 mm<sup>3</sup>) and MOD (>85 mm) (Table IV).

The univariate analysis (Table IV) shows the ORs and their 95% CI computed from 2 × 2 contingency tables, with each of the four Day 3 cut-off values as the predictor and OHSS occurrence as the dependent



**Table III** Areas under the receiver characteristic operating curve, sensitivity, specificity and optimal cut-offs of Day 0 and Day 3 parameters for severe OHSS prediction.

	AUC	95% CI		Cut-off <sup>a</sup>	P-value	Sensitivity %	Specificity %
<b>Day 0</b>							
Number of oocytes retrieved	0.70	0.62	0.78	>25	<0.001	67.4	62.7
Ht <sub>D0</sub>	0.60	0.54	0.65	>38.3	0.045	65.1	52.2
WBC <sub>D0</sub>	0.65	0.59	0.70	>11 200	0.002	46.5	76.8
Ascites grade <sub>D0</sub>	0.51	0.45	0.57	>1	0.887	4.7	97.1
<b>Day 3</b>							
Ht <sub>D3</sub>	0.72	0.67	0.77	>39.2	<0.001	93.0	48.2
WBC <sub>D3</sub>	0.75	0.70	0.80	>12 900	<0.001	74.4	71.4
Ascites grade <sub>D3</sub>	0.88	0.84	0.92	>2	<0.001	76.7	85.5
MOD <sub>D3 (mm)</sub>	0.74	0.69	0.78	>85	<0.001	65.1	73.7
<b>Changes between Day 3 and Day 0</b>							
cHt <sub>D3-D0</sub>	0.63	0.58	0.69	>2.1	0.013	62.8	61.2
cWBC <sub>D3-D0</sub>	0.68	0.62	0.73	>2050	<0.001	76.7	54.7
cAsc <sub>D3-D0</sub>	0.88	0.83	0.91	>1	<0.001	76.7	86.6
<b>Percentage changes between Day 3 and Day 0 (%)</b>							
cHt <sub>D3-D0</sub>	0.63	0.57	0.68	>4.2	0.013	67.4	53.6
cWBC <sub>D3-D0</sub>	0.61	0.56	0.67	>11.3	0.027	83.7	38.0

<sup>a</sup>Calculated as the maximum value of the Youden index = Sensitivity + Specificity – 1.

Ht indicates haematocrit; WBC, white blood cell count; MOD, maximal ovarian diameter; cHt, change of haematocrit; cWBC, change of white blood cell count; cAsc, change of ascites grade.

**Table IV** Odds ratios with their 95% CI computed with logistic regression models of the four Day 3 predictors for the occurrence of early OHSS.

Parameter	Using cutoff values						
	Cutoff	Univariate analysis		Multivariate analysis		Multivariate analysis using continuous variables	
		OR	95% CI	OR	95% CI	OR	95% CI
Ascites	>2	19.6	9.0–43.0	18.4	7.3–46.2	8.6	4.4–17.1
Ht	>39.2%	12.2	3.7–40.5	7.8	2.0–30.3	1.1	0.9–1.3
WBC	>12 900/mm <sup>3</sup>	7.3	3.5–15.3	5.4	2.2–13.6	1.0	1.0–1.0
MOD	>85 mm	5.2	2.7–10.4	4.2	1.7–10.3	1.1	1.0–1.1

OR indicates odds ratio.

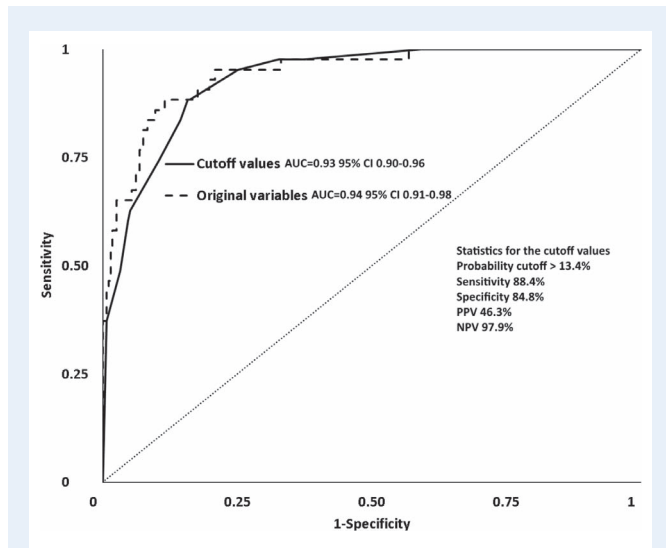
The analysis for the cutoff values was performed both using univariate and multivariate analysis, while for the original variables, multivariate analysis was used.

variable. All the ORs were statistically significant. In particular, the OR = 19.6 for ascites >2 means that the odds of the number of women who will develop OHSS to the number of women who will not develop OHSS is 20 times greater among women who at Day 3 have ascites grade >2 than among women who have ascites grade ≤2.

The multivariate analysis (Table IV) shows that all four predictors retain their statistical significance and they all enter into the stepwise logistic regression model. This means that each of the four Day 3 predictors carries independent and additive information for the prediction of the manifestation of OHSS.

Based on the results of the stepwise logistic regression from the previous step, a final prediction model was constructed. The model using the cut-off values had an AUC of 0.93, 95% CI of 0.90–0.96, a cut-off of predicted probability >13.4%, with a sensitivity of 88.4%, a specificity of 84.8%, a positive predictive value of 46.3% and a negative predictive value of 97.9% (Fig. 2). In comparison, ROC analysis using the continuous variables for ascites grade, Ht, WBC and MOD yielded an AUC of 0.94, a 95% CI of 0.91–0.98 (Fig. 2).

It must be noted that the construction of different models was attempted, including more variables measured on Day 0, as well as changes of Ht and WBC between Day 0 and Day 3 (results not shown).



**Figure 2** Receiver operating characteristic curves showing the discrimination ability of the logistic regression equation to predict the occurrence of severe early ovarian hyperstimulation syndrome. Curves were generated using either the cut-off values or the continuous (original) variables, which were ascites grades, maximal ovarian diameter (MOD), haematocrit (Ht), white blood cell (WBC) count. PPV indicates positive predictive value; NPV, negative predictive value.

However, inclusion of more variables did not improve the predictive ability of the model. The inclusion of the four variables (ascites grade, Ht, WBC and MOD) measured on Day 3 produced the simplest and most predictive model.

The cutoff probability of 13.4% predicted by the model coincides with the observed probability of OHSS occurrence ( $43/321 = 13.4\%$ ). For the majority of the women, there are  $\leq 2$  criteria over the cut-off on Day 3 (81% of women, Table V); hence, the predicted probability of severe OHSS according to the equation is  $< 13.4\%$ . Once the number of criteria over the cut-off increases, the predicted probability becomes greater than the observed one.

Table V shows the predicted incidence of Day 5 severe OHSS depending on the number of criteria fulfilled on Day 3.

The probability of severe OHSS with no criteria fulfilled on Day 3 is 0%, with one criterion is 0.8%; with two criteria, 13.3%; with three criteria, 37.2%; and with four criteria, 88.9%. Subgroup analysis of the category with three criteria showed that when ascites grade  $\geq 3$  is among the criteria, then the probability of severe OHSS increases to 47.8%, while if ascites is not included in the criteria, then the probability of severe OHSS is reduced to 25.0% (Table V).

Accordingly, a decision-making algorithm for performing embryo transfer in high-risk patients is proposed (Fig. 3).

## Discussion

The present study describes the predictive value of several early luteal-phase markers associated with OHSS, such as ascites grade, Ht, WBC count and MOD, in women at high risk for severe OHSS receiving hCG for triggering of final oocyte maturation. Using these criteria, a novel and highly accurate decision-making algorithm for performing

**Table V** Probability of severe OHSS on Day 5 depending on the number of Day 3 parameters over the specified cut-offs\*.

Number of parameters over the cut-off on Day 3	Number of patients	Probability of severe OHSS on Day 5 (n) 95% CI
0	66	0 (0) 0–5.5
1	119	0.8 (1) 0.15–4.6
2	75	13.3 (10) 7.4–22.8
3 (overall)	43	37.2 (16) 24.38–52.1
3 (not inclusive of ascites)	20	25 (5) 11.19–46.9
3 (inclusive of ascites)	23	47.8 (11) 29.24–67.0
4	18	88.9 (16) 67.2–98.1

\*Ascites grade  $> 2$ , Ht  $> 39.2\%$ , WBC  $> 12\,900\text{ mm}^3$  and MOD  $> 85\text{ mm}$ .

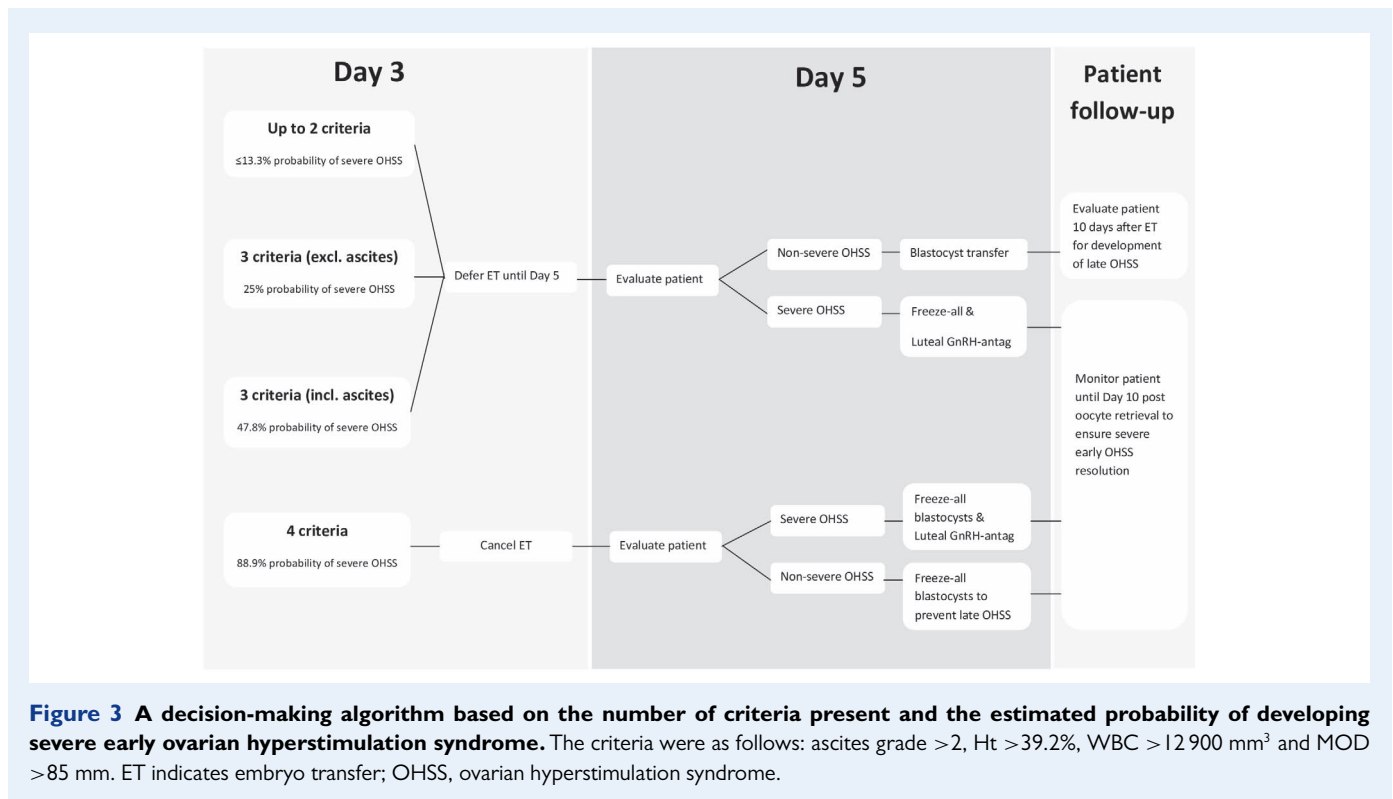
embryo transfer was constructed, based on the predicted probability of subsequent severe OHSS development.

While early OHSS predictors, such as anti-Müllerian hormone and antral follicle count, are very important for selecting the optimal controlled ovarian stimulation protocol and mode of triggering final oocyte maturation (Navot *et al.*, 1996; Papanikolaou *et al.*, 2006; Nastri *et al.*, 2015; Griesinger *et al.*, 2016), they can have limited value once hCG has been administered, either because of the patient's wish or due to the use of GnRH agonists for downregulation. In these cases, the first days of the luteal phase after hCG and oocyte retrieval are very crucial for patients with high oocyte yields. In a recent study, we showed that careful monitoring of ultrasound parameters (ascites grade and MOD) and haemoconcentration markers (Ht and WBC) in the early luteal phase may provide useful information regarding the evolution of OHSS in these patients (Lainas *et al.*, 2018). In some cases, the syndrome is aggravated, leading to severe or even life-threatening OHSS (Confidential Enquiry into Maternal and Child Health, 2007; Braat *et al.*, 2010).

The present study suggests for the first time that ascites grade, Ht, WBC and MOD, assessed 3 days following oocyte retrieval, can predict development of severe OHSS 2 days in advance in high responders triggered with hCG. Using ROC curve analysis, we propose specific cut-offs for the absolute values of the aforementioned parameters on Day 3, as well as their relative changes between Day 0 and Day 3.

The predictive model developed, based on the cut-offs of Day 3 parameters (ascites, Ht, WBC and MOD), provided high predictive ability (0.93 AUC, 88.5% sensitivity, 84.2% specificity) to identify patients that will develop severe OHSS.

Generally, in all patients at high risk for OHSS, culture to the blastocyst stage is preferable to cleavage stage embryo transfer since this not only makes it feasible to select the best embryos for transfer but also allows better evaluation of the patient, with the possibility of



embryo transfer cancellation, in case severe OHSS develops by Day 5. Even patients with no, or one parameter, over the cut-off on Day 3, who have a minimal probability ( $\leq 0.8\%$ ) of severe OHSS, should benefit from extended embryo culture for the reasons mentioned above (Fig. 3).

According to our decision-making algorithm, high risk for OHSS patients with three criteria over the cutoff on Day 3 have an overall 37.2% probability of severe OHSS. This probability is further increased to 47.8% if ascites grade  $\geq 3$  (moderate ascites) is among the criteria. These patients are at considerable risk of developing severe OHSS by Day 5. It is suggested that embryo transfer on Day 3 should be avoided and embryos are cultured until the blastocyst stage. The patient must be examined again (ascites, MOD, Ht, WBC) on Day 5 in order to verify that OHSS is regressing. Blastocyst transfer may be performed if severe OHSS is not diagnosed on Day 5. However, patients should be informed about the probability of late-onset OHSS (1.1%; Lainas et al., 2012) in case of a successful pregnancy (Fig. 3) and be offered a freeze-all option.

Patients at high risk for OHSS with all four criteria (ascites, MOD, Ht, WBC) over the cut-off on Day 3 are almost certain (88.9%) to develop severe OHSS by Day 5. These patients should be offered extended counselling regarding the potential complications, as well as management options of severe OHSS. Specifically, embryo transfer cancellation and cryopreservation of all embryos are mandatory for these patients. Upon diagnosis of severe OHSS, the clinician and the patients could consider administration of luteal GnRH antagonist, which has been proposed as a tertiary OHSS prevention, at an outpatient level (Griesinger, 2010; Lainas et al., 2012) (Fig. 3).

Similar to previous findings (Asch et al., 1991; Navot et al., 1996), in the present study, the number of oocytes retrieved was significantly associated with the probability of severe early OHSS development and may be used as a useful parameter on the day of oocyte retrieval, alerting the clinician to closely monitor the patient for the development of severe OHSS (Lainas et al., 2012).

Today, it is possible to make OHSS a complication of the past and the recommended approach for high-risk patients is the use of GnRH antagonist protocols and GnRH agonist trigger (Devroey et al., 2011). Nevertheless, GnRH agonist protocols and hCG trigger are still widely used in the general IVF population (De Ziegler and Shoham, 2013, Tur-Kaspa and Fauser). This means that a large proportion of high-risk patients are still being triggered with hCG, either due to inaccurate estimation of follicle numbers, inexperience in the use of antagonist protocols and agonist trigger, clinic scheduling, insufficient patient monitoring or inadequate estimation of OHSS risk. Therefore, the decision-making tool proposed in the current study is of particular relevance for the clinical management of these patients and may be used to filter out patients in whom embryo transfer should be cancelled in view of imminent development of severe early OHSS. This approach is important also for the prevention of late pregnancy-induced OHSS, for which only symptomatic therapy exists.

It must be emphasized that the availability of the present decision-making algorithm should in no way be seen as a way of encouraging the use of hCG trigger in patients at high risk for OHSS. On the contrary, every measure should be taken to eliminate the development of OHSS. This can be achieved by treatment segmentation, using GnRH antagonist protocols, GnRH agonist trigger and elective embryo



cryopreservation, according to the OHSS-free clinic concept (Devroey *et al.*, 2011).

Accurate prediction/diagnosis of severe early OHSS after careful patient monitoring should automatically lead to embryo transfer cancellation to prevent late OHSS. This is an important decision for patient safety, as in many cases the development of late OHSS may occur after embryo transfer in patients with undiagnosed severe early OHSS (Lainas *et al.*, 2012).

The present study describes, for the first time, a decision-making tool that can significantly help clinicians decide whether it is safe to proceed with embryo transfer or freeze all embryos in patients at high risk for OHSS who have been triggered with hCG instead of GnRH agonist. The tool is highly predictive of severe OHSS on Day 5 and is simple to use, requiring Day 3 measurements of ascites grade, MOD, Ht and WBC. It should be noted that, in high-risk patients triggered with hCG, the use of a decision-making algorithm should be backed by the availability of tertiary OHSS prevention using GnRH antagonist administration in the luteal phase and cryopreservation of all embryos (Lainas *et al.*, 2012), in case severe early OHSS does develop.

The present study is limited by its retrospective nature. Therefore, it cannot be excluded that non-apparent sources of bias might be present. In addition, we acknowledge the lack of external validation of our model. We have created a web-based calculator (<http://ohsspredict.org>), which is based on the present algorithm, for wider access and usage of our tool. By inserting the values of ascites grade, MOD, Ht and WBC of high-risk patients on Day 3 post-oocyte retrieval, the clinician instantly receives the predicted probability of severe OHSS development on Day 5.

In conclusion, despite current recommendations on the use of GnRH agonist trigger in patients at high risk for OHSS, a considerable proportion of clinicians still use the long or antagonist protocol combined with hCG trigger. As a result, OHSS is still present and affects a significant number of women undergoing IVF worldwide. The present decision-making tool may help clinicians make the best choices for their patients by considering timely OHSS-preventive interventions, such as transfer cancellation and freezing all embryos. The ultimate goal is the accurate diagnosis of severe early OHSS in women triggered with hCG and elimination of pregnancy-induced late OHSS, for which only symptomatic therapy exists.

## Acknowledgements

The authors wish to thank Dr I.Z. Zorzovilis for assisting with clinical work, Mrs G. Stavropoulou for patient coordination and Mrs M. Panagopoulou, E. Giouvani, S. Ntovolou, E. Lamprou and G. Kalogeropoulou for data entry.

## Authors' roles

G.T.L. and T.G.L. had the original conception of the study, participated in study design, acquisition and interpretation of data and writing and revision of the manuscript and performed clinical work. I.A.S. participated in the acquisition and interpretation of data, wrote and revised the manuscript and performed embryology work. C.A.V. and M.K. contributed to the statistical analysis and interpretation

of data and participated in the revision of the manuscript. B.C.T. and G.K.P. participated in the interpretation of data and revision of the manuscript. E.M.K. participated in study design, acquisition and interpretation of data and revision of the manuscript and performed the statistical analysis. All authors approved the final version of the manuscript.

## Funding

NHMRC Early Career Fellowship (GNT1147154) to C.A.V.

## Conflict of interest

All authors declare that they have no conflict of interest related to the present study.

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