Known genetic alterations are recognised as an important detrimental factor in male reproductive health, accounting for roughly 3% of all causes of male infertility [1]. Overall, 3–19% of infertile men are diagnosed with a chromosomal abnormality [2]. Both sex and autosomal chromosome alterations can be found in infertile individuals, though
their specific clinical meaning is not always fully clear [2]. Current European Association of Urology (EAU) guidelines recommend karyotype analysis (KA) in men with either azoospermia or oligozoospermia (ie, sperm concentration < 10 million/ml), and in case of a family history suggestive for recurrent spontaneous abortions, malformations, or mental retardation [1]. Only a few studies have supported this indication [3], and also, the EAU guidelines have never been validated to date. Moreover, the choice of a specific cut-off for a continuous variable (ie, < 10 million/ml for sperm concentration) may represent an important limitation. Indeed, the relationship between sperm concentration and risk of KA alterations may be either linear or exponential, and the categorisation of patients into two rough groups may importantly reduce the diagnostic process accuracy.

To address these issues, we aimed at validating the EAU guidelines using a homogenous cohort of White European men presenting for primary couple’s infertility, and to develop a novel model predicting karyotype alterations.

We analysed data from 1168 consecutive infertile men assessed at a single academic centre between September 2005 and January 2015. According to the World Health Organisation criteria, infertility was defined as not conceiving a pregnancy after at least 12 mo of unprotected intercourse [4]. Male factor infertility was defined after a comprehensive diagnostic evaluation of the female partners. Two cohorts were analysed: (1) a validation cohort (VC; N = 1168) for the EAU guideline validation, (2) a nomogram cohort (NC; N = 760) with complete clinical and laboratory data for the development of a novel predictive model. As for our internal guidelines which contemplate a comprehensive metabolic, hormonal, and genetic assessment for each evaluated infertile man, a KA was also requested for every patient during the clinical workup. The EAU guidelines were therefore retrospectively validated in the VC cohort with the estimation of their sensitivity, specificity, and discrimination. Thereafter, the NC cohort was used to develop a logistic regression-based nomogram predicting alterations at KA. Predictors consisted of patient sperm concentration, luteinising hormone (LH), and mean testicular volume (assessed through a Prader orchidometer). Furthermore, the relationship between sperm concentration and alterations at KA was graphically explored using the locally-weighted scatter-plot smoothing (lowess) method. LH and mean testicular volume were identified as the most informative variables among the endocrine and the exocrine compartment, respectively. The area under the receiver operating characteristic-curve was used to quantify discrimination of the novel predictive model. The model was then subjected to 200 bootstrap resamples in order to reduce overfit bias. The gain in predictive accuracy was quantified, and areas under the curve were compared using the Wald test. Finally, we used decision-curve analysis to evaluate the clinical net-benefit of the two predictive models.

Descriptive characteristics of patients are shown in Supplementary Table 1. Median (interquartile range) age of the patients was 36 (33–40) yr. Alterations at KA were found in 60 (5.1%) and 33 (4.3%) of the VC and the NC individuals, respectively. Overall, 742 (64%) patients would have deserved KA according to the EAU guidelines. Of those, 48 (6.9% of the assessable patients according to EAU guidelines) actually displayed any kind of alteration at KA. Conversely, 12 (20%) out of 60 patients with karyotype abnormalities would not have been candidates for the genetic assessment based on the EAU guidelines criteria. Overall, 694 (63%) patients would have been candidates for genetic workup despite having a normal karyotype. As a whole, the EAU guidelines sensitivity, specificity, and discrimination were 80%, 37%, and 59%, respectively. Table 1 shows logistic regression analysis predicting the presence of any karyotype abnormality in the cohort used to develop our model. The associated nomogram is illustrated in Figure 1A. The decision curve analysis (Fig. 1B) illustrates that our predictive model provides a higher clinical net-benefit compared with the EAU guidelines. The calibration plot (Fig. 1C) shows the predicted probabilities plotted against the observed KA rate.

Though EAU guidelines provide clinicians with a clear-cut recommendation for KA in infertile men, our data indicate that their application results in a loss of diagnosed alterations along with an overtreatment. KA is actually an expensive test, both economically and psychologically. However, it is fundamental considering its consequences in terms of etiologic assessment and for subsequent genetic counselling. Existing data account for increased gonadotropin levels and higher azoospermia rate in men with karyotype abnormalities, without any correlation between them and sperm parameters [5]. Of importance, our results confirm higher LH and follicle-stimulating hormone (FSH) values in men with confirmed alterations at KA (Supplementary Table 1); in spite of this, we decided not to include FSH values in our model since it would not have added anything in terms of both clinical (sperm concentration is a better proxy of testicular function than FSH itself) and statistical significance (p > 0.05 at Wald test between our model with and without FSH). Moreover, using the lowess method, the role of sperm concentration appears rather complex to be limited to the azoospermia category (Supplementary Fig. 1). Although specifically designed for the male infertility setting, EAU guidelines, relying mainly on sperm concentration, are somehow reductive considering the whole clinical scenario a karyotype alteration might eventually prompt. If some karyotype alterations might be

![Table 1](image.png)

### Table 1 – Logistic (odds ratio; p value [95% confidence interval]) regression models predicting karyotype analysis (total testosterone < 3ng/ml) in the nomogram cohort

<table>
<thead>
<tr>
<th></th>
<th>UVA</th>
<th>MVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH</td>
<td>1.22; &lt;0.001 (1.15–1.31)</td>
<td>1.18; &lt;0.001 (1.09–1.27)</td>
</tr>
<tr>
<td>Mean testis size (Prader)</td>
<td>0.87; &lt;0.001 (0.81–0.93)</td>
<td>0.98; 0.53 (0.91–1.05)</td>
</tr>
<tr>
<td>Sperm concentration</td>
<td>0.95; 0.01 (0.92–0.99)</td>
<td>0.97; 0.12 (0.94–1.01)</td>
</tr>
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</table>

LH = luteinising hormone; MVA = multivariable analysis; UVA = univariable analysis.
Fig. 1 – (A) Logistic regression based nomogram predicting the probability of alterations at karyotype analysis based on luteinising hormone (LH) values, mean testis volume, and sperm concentration. Specificity, sensitivity, positive predictive value (PPV), and negative predictive value (NPV) are...
clinically insignificant, whenever this would not be the case they emerge as multifaceted clinical syndromes, wherein infertility is just one of the several features (e.g., Klinefelter syndrome) [6]. In this regard, an accurate diagnosis of the underlying karyotype alterations is of paramount importance for at least three reasons: (1) it provides psychological relief clarifying a possible cause of the infertility, (2) in the context of clinical syndromes, it might anticipate future or yet occult health issues which would otherwise progress unnoticed in infertile, and therefore often young men, and (3) genetic counselling might actually give an appraisal of the risk of transmission of the known alteration to the offspring. Our nomogram tries to overcome EAU guidelines’ lack of diagnostic power by more comprehensively evaluating the clinical features of infertile men. Excluding from KA men with a nomogram-calculated probability < 2%, 519 (68.3%) of men would deserve KA, with only two (6.1% of the positive KA group) missed evaluations. Remarkably, those two missed patients had alterations deemed as clinical insignificant by our medical geneticist. Although not specifically reducing the risk of overtreatment, our nomogram avoids a 20% diagnostic loss of possible KA in infertile men brought by the EAU guidelines application. We understand how the use of a nomogram might be more challenging compared with standardised cut-offs, but we are highly confident that stronger diagnostic efforts are of considerable importance since up to 40% of male infertility cases still remain unexplained, and thereafter not adequately counselled.

Author contributions: Andrea Salonia had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Ventimiglia, Salonia.
Acquisition of data: Capogrosso, Boeri, Pederzoli, Cazzaniga, Scano, Ippolito.
Analysis and interpretation of data: Ventimiglia, Salonia.
Drafting of the manuscript: Ventimiglia, Salonia.
Critical revision of the manuscript for important intellectual content: Alfano, Salonia, Montorsi.
Statistical analysis: Ventimiglia, Capogrosso, Fossati.

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Supervision: Salonia, Montorsi.
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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.eururo.2016.06.015.

References


reported for the analysed probability thresholds. The predictive accuracy for the nomogram after 200 bootstrap resamples was 72% (p = 0.02 compared with European Association of Urology guidelines). (B) Decision-curve analyses demonstrating the net benefit associated with the use of the nomogram (orange dashed line) in detecting karyotype alterations during male infertility work-up (i.e., for each value of threshold probability the nomogram has a higher net benefit compared with the European Association of Urology guidelines, represented by the orange dashed line); the continuous line represents the karyotype work-up of all patients. (C) Calibration plot showing predicted probabilities against the observed karyotype abnormalities rate. The dashed line indicates the location of the ideal nomogram, in which predicted and actual probabilities are identical. The dotted line indicates the actual nomogram performance, whereas the solid line represents the performance of the bootstrap model. Prob. = probability.