Evaluation of Measurement Uncertainty of First Trimester Prenatal Screening

Birinci Trimester Prenatal Tarama Testinde Ölçüm Belirsizliğinin Değerlendirilmesi

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ABSTRACT

Background: First trimester prenatal screening gives the risk rates associated with aneuploidies. Measurement uncertainty is defined as a magnitude showing distribution of the measured values. The aim of this study is to investigate the effects of measurement uncertainty on prenatal screening and compare two different measurement uncertainty guidelines which suggest different equations.

Material and Methods: This retrospective study was performed with results of 544 patients. Uncertainties of free-βsubunit human Chorionic Gonadotropin (free β-hCG) and Pregnancy Associated Protein-A (PAPP-A) were calculated as defined by AACB and Nordtest guides.The best-case and the worst-case scenarios were created for risk rates of trisomies. New risks were recalculated by adding and subtracting uncertainty values from free β-hCG and PAPP-A.

Results: The number of patients who have a risk rate >1:1500 and to be subject to further investigation was 58. This number decreased to 38 and 36 with best-case scenarios, while the number increased to 94 and 99 with worst-case scenarios with the uncertainty values obtained from the AACB and Nordtest guidelines, respectively ($P<0.005$). There was a significant difference between median risks of the patients with two guidelines with best-case and the worst-case scenarios($P<0.005$).

Conclusions: When a result is calculated with multiple parameters, calculation of uncertainty and reporting with the result may significantly affect the outcome. The measurement uncertainty equation to be selected is also important.

Key words: Prenatal Screening; Uncertainty; HCG-beta; PAPP-A

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ÖZET

Amaç: Birinci trimester prenatal tarama, anöploidiler ile ilişkili risk oranlarını verir. Ölçüm belirsizliği ise, ölçülen değerlerin dağılımını gösteren bir büyüklük olarak tanımlanır. Bu çalışmanın amacı, ölçüm belirsizliğinin doğum öncesi taramaya etkilerini araştırmak ve farklı denklemler öneren iki farklı ölçüm belirsizliği kılavuzunu karşılaştırmaktır.

Gereç ve Yöntem: Bu retrospektif çalışma 544 hastanın sonuçlarıyla yapıldı. Serbest beta Koryonik Gonadotropin (serbest β-hCG) ve Gebelik İlişkili Protein-A (PAPP-A) belirsizlikleri AACB ve Nordtest kılavuzları tarafından tanımlandığı şekilde hesaplandı. Trizomi risk oranları için en iyi ve en kötü durum senaryoları oluşturuldu. Serbest β-hCG ve PAPP-A'nın belirsizlik değerleri mevcut değerlere eklenerek ve çıkarılarak yeni riskler yeniden hesaplandı.

Bulgular: Risk oranı> 1: 1500 olan ve ileri incelemeye tabi tutulacak hasta sayısı 58 idi. Bu sayı sırasıyla AACB ve Nordtest kılavuzları ile hesaplanan sonuçlarla, en iyi senaryolarda 38 ve 36'ya düşerken, en kötü senaryolarla 94 ve 99'a yükseldi (P <0.005). En iyi durum ve en kötü durum senaryolarına sahip iki kılavuza sahip hastaların medyan riskleri arasında anlamlı bir fark vardı (P <0.005).

Sonuç: Ölçüm belirsizliğinin sonuçla birlikte raporlanması klinik kararı etkileyebilir. Çalışmamızda olduğu gibi bir sonuç birden fazla parametre ile hesaplandığında, belirsizliğin hesaplanması ve sonuçla birlikte raporlanması sonucu önemli ölçüde etkileyebilir. Seçilecek ölçüm belirsizliği denklemi de önemlidir.

Anahtar sözcükler: Tarama; Belirsizlik; HCG-beta; PAPP-A

INTRODUCTION

First trimester prenatal screening is a widely used screening method for autosomal trisomies. Most commonly used protocol in first trimester screening involves measuring of nuchal translucency (NT) by ultrasonography and measuring of free β-subunit human chorionic gonadotropin (free β-hCG) and pregnancy associated protein- A (PAPP-A) from maternal blood. It is performed between 11 and 14 weeks of gestation (1). Although prenatal screening does not give definite results, it gives risk rates associated with trisomy 21 (Down Syndrome), trisomy 13 (Patau Syndrome) and trisomy 18 (Edwards Syndrome). NT, PAPP-A and free β-hCG measurements are expressed as multiples of gestational age-specific medians (MoM - Multiples of Medians). Higher free β-hCG and lower PAPP-A values are associated with Down Syndrome and lower values of both parameters are related to both of trisomy 18 and trisomy 13. Detection rate of trisomy 21 by the combination of maternal age, NT of fetus, PAPP-A and free β-hCG is 64-70% with a false positive rate of 5%–8% (1-4). Patients who has risk more than 1:1500 undergo further investigations which are ultrasound examination of the nasal bone and tricuspid regurgitation or doppler velocity waveforms in the ductus venosus, and chorionic villus sampling (CVS) as an invasive prenatal testing for aneuploidy (5-7).

In the International Vocabulary of Metrology (VIM), the uncertainty of measurement is defined as a magnitude showing the distribution of the measured values (8). (available at: http://redsang.ial.sp.gov.br/site/docs_leis/im/im6.pdf). In other words, measurement uncertainty indicates a range of values which covers the exact value of the measured parameter (9).

ISO Technical Specifications 20914 based on intermediate precision results and calibration uncertainty and recommends the correction of bias or the inclusion bias uncertainty in the uncertainty calculation (10). However, as in our study, many calibrators do not contain values related to measurement uncertainty yet.

Many methods have been defined for the calculation of measurement uncertainty. These methods can be divided into two main approaches, bottom-up and top-down (11,12). Australasian Association of Clinical Biochemists Uncertainty of Measurement Working Group (AACB) and Nordtest Guideline

(Handbook For Calculation of Measurement Uncertainty in Environmental Laboratories) are the most commonly used guides written according to the top to bottom approach. According to the AACB, calculation of measurement uncertainty requires at least 6 months of IQC data and at least 30 data per level. Measurement uncertainty equation is '$1.96xCV\%_{IQC}$' (13).

The Nordtest guideline proposes a range of equations using a combination of CV of internal quality control (IQC) with participants numbers, CV and bias values of external quality control (EQC) (14). The average of the bias values (RMSbias), where the "n" is the number EQCs, is found with the equation '$\sqrt{\frac{\sum(bias)^2}{n}}$' Arithmetic mean of CV% and participant numbers (pn) of EQCs are used for calculation of u(Cref) as '$\frac{CV\%EQC}{\sqrt{pn\ EQC}}$'. All these variables are combined in the following equation:

Measurement uncertainty
$=2x\sqrt{(IQC\ CV\%)^2 + RMSbias^2 + u(Cref)^2}$

Laboratory results play an important role in diagnosis and treatment. These results are evaluated according to the reference values or cut off values. If the measurement uncertainty is reported with the result, a patient result which is less than cut off value may become partially higher than the cut off value. This may lead to changes in clinical decision (15).

The aim of this study is to investigate the effects of measurement uncertainty on the first trimester prenatal screening by comparing the reported patient results with other possible results. We aimed to reveal the importance of measurement uncertainty guideline to be used also.

MATERIALS AND METHODS

Subjects: This retrospective study was performed by recalculating the screening results of 544 pregnant women who had first trimester maternal screening in our hospital in 2017. Patients older than 14 weeks of gestation were excluded from the study. We used Randox Maternal Screening as EQC monthly (Cycle9, Sample1-12), and Randox Maternal Control as daily IQC in accordance with manufacturers recommendations.

Methods: Measurement uncertainties of free β-hCG and PAPP-A were calculated as defined by AACB and Nordtest guides for our autoanalyzer (Siemens immulite XPi 2000, Walpole/USA) with 2017 data. Both of guides recommend to calculate measurement uncertainty for different levels separately. So we have used IQC mean values stated by the manufacturer as our reference levels. For Nordtest guide we classified EQC samples according to their mean levels, thus, we divided the EQC samples into three groups (low, medium, high) in accordance with the three levels of IQC.

Patients were categorized according to their PAPP-A and free β-hCG values and named as low, medium, high groups (Table 1). Different scenarios had been created for the study (Table 2). Uncertainty values were added to the present PAPP-A and free β-hCG results to obtain the possible maximum results. The uncertainty values were also subtracted from the present results for possible minimum results. Patients' uneuploidy risks were recalculated by PRISCA 5.0 Prenatal Risk Calculation Software by using of new PAPP-A and free β-hCG values. NT of fetus; age, smoking habits, race and weight of mother, gestational age, diabetes mellitus and IVF situations were not changed.

More than one type of risk is calculated for trisomy 21. Biochemical risk is calculated by using of maternal age, PAPP-A and free β-hCG values. Combined risk is calculated by using of maternal age, PAPP-A and free β-hCG values and fetal NT. In our study, we examined the effect of measurement uncertainty on both risk types.

Table-1. IQC mean values, calculated measurement uncertainity % results and number of patients in the range
Tablo 1: IQC ortalama değerleri, hesaplanan ölçüm belirsizliği % sonuçları ve aralıktaki hasta sayısı

	Levels of IQC[a]	Mean values of IQC[a]	Covered range[b]	AACB MU %[c]	Nordtest MU % [d]	Number of patients
PAPP-A	Low	1.89 IU/L	<5.50 IU/L	10.94	11.21	473
	Medium	9.11 IU/L	5.50-12.6 IU/L	15.36	23.01	64
	High	16.1 IU/L	>12.60 IU/L	18.12	15.21	7
free β-hCG	Low	17.50 μg/L	<33.90 μg/L	18.24	18.2	312
	Medium	50.3 μg/L	33.90-78.65 μg/L	18.58	18.55	192
	High	107 μg/L	>78.65 μg/L	14.74	23.77	40

[a]: Internal quality control
[b]: Consideration range of internal quality control material
[c]: Measuremet uncertainty percentages calculated with Australasian Association of Clinical Biochemists guideline
[d]: Measuremet uncertainty percentages calculated with Nordtest guideline

Table 2. Algorithm for creating scenarios
Tablo 2: Senaryo Algoritmaları

Scenarios[a]	PAPP-A	free β hCG
The worst case scenarios of trisomy 21	possible minimum result[b]	possible maximum result[c]
The best case scenarios of trisomy 21	possible maximum result[c]	possible minimum result[b]
The worst case scenarios of trisomy 13/18	possible minimum result[b]	possible minimum result[b]
The best case scenarios of trisomy 13/18	possible maximum result[c]	possible maximum result[c]

[a]: Scenarios calculated with *measurement uncertainty obtained from* Australasian Association of Clinical Biochemists guideline and Nordtest guideline seperately
[b]: *Possible minimum results calculated by subtracting of measurement uncertainty values from present patient results*
[c]: *Possible maximum results calculated by addition of measurement uncertainty values to present patient results*

The risk rates were calculated eight times for each patient. Scenarios were named according to used guideline (e.g. The best-case scenario trisomy 21 AACB). The minimum and the maximum risks that Prisca System can calculate are <1:10000 and >1:50. Results of <1: 10000 and >1:50 were considered to be 1:10000 and 1:50 since the exact values were not known.

Statistical analysis: SPSS 23.0 program (IBM Corp. Released 2015.IBM SPSS Statistics for Windows, NY: IBM Corp.) was used for statistical analysis. Reported and subsequently calculated risk rates, PAPP-A and free β-hCG values analyzed with Kolmogorov Smirnov test to evaluate distrubution. Wilcoxon signed rank test is used for comparing median values of risk rates. McNemar test is used for comparing the number of patients with changing risk categories. P<0.05 accepted as statistically significant.

RESULTS

Manufacturer recommendation for CV% for PAPP-A and free β-hCG differs between 3.5-12% and 6.5-11.3%, respectively. In our study CV% of IQC of PAPP-A were between 5.47-9.06% and free β-hCG were between 7.37-9.29%.

The risk rates obtained by all scenarios and reported risk rates, free β-hCG and PAPP-A values showed nonparametric distribution. For reported results, median value and (interquartile range - IQR) of PAPP-A was 2.65 (2.63); median and (IQR) of free β-hCG was 30.8 (26.5). Median and (IQR) of PAPP-A MoM was 0.86 (0.69); median and (IQR) of free β-hCG MoM was 0.79 (0.65). Mean age was 30.75±5.3 years.

IQC levels of PAPP-A and free β-hCG and calculated measurement uncertainity percentages are showed in Table-I. When

biochemical risk of trisomy 21 recalculated for four scenarios, results were statistically different from reported results (Graph-1). Also, there were significant differences between guidelines for both of the best-case and worst-case scenarios (P<0.001).

Because of its higher detection rate, combined risk is more preferable than biochemical risk and using of NT alone. Prisca system recommends 1:250 as a high risk cut off. 19 patients were reported as have risk rates more than 1:250. According to the worst-case scenarios of AACB Nordtest and these numbers increased to 25 and 26 (P=0.031 and P=0.016 respectively). According to the best-case scenarios of AACB and Nordtest guideline these numbers decreased to 12 and 9 (P=0.016 and P=0.002 respectively).

Table 3 shows the number of patients to be subject to further investigation, based on different scenarios.

328 of 544 patients combined trisomy 21 risks reported as <1:10000. Therefore median of reported combined risk is <1:10000. The results of these patients did not change when recalculated according to the best-case scenarios as expected. On the other hand according to the worst-case scenarios of AACB and Nordtest guideline this number decreased from 328 to 250 and 248 respectively (P < 0.001). Median of combined risks of trisomy 21 in best and worst-case scenarios were significantly different from reported trisomy 21 risk calculated with both of the AACB and Nordtest guidelines (P<0.001). There are also a significant differences between guidelines for both best and worst scenarios (P<0.001).

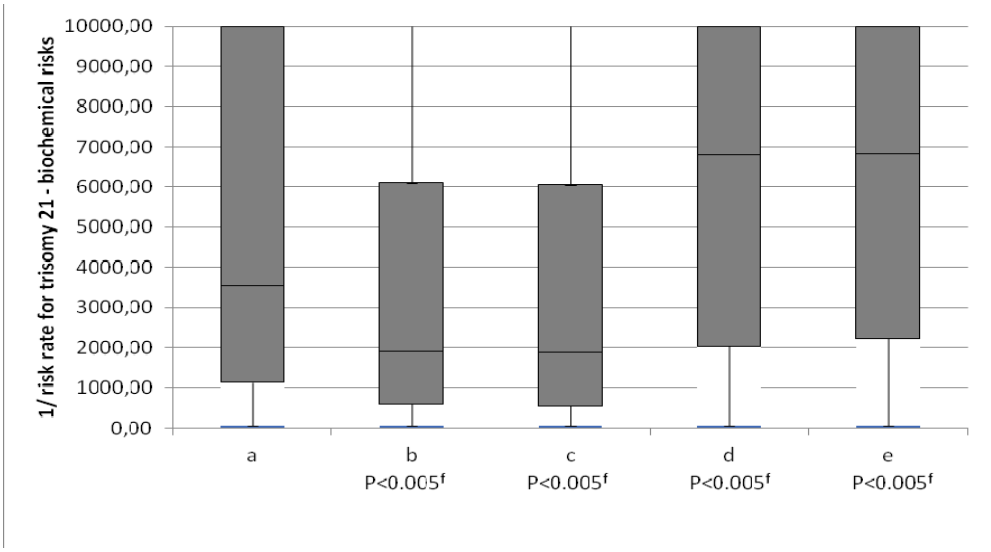
5 of 544 patients combined trisomy 21 risks reported as >1:50, according to the worst-case scenarios with AACB and Nordtest this number increased to 8 and 9; and for the best-case scenarios this number decreased to 3 and 4 respectively (P > 0.05).

The changes in recalculated results of patients with a result of <1:10000 and >1:50 depends on how far the exect results are from these values. The difference between the median values increased in the statistics excluding these patients. The results are given in Graph 2. There were also significant differences between guidelines for both of the best-case and worst-case scenarios (P<0.001).

Table 3. Number of patients who have combined risk for trisomy 21 as <1:1500 and >1:1500 according to different scenarios and P values.
Tablo 3. Farklı senaryolara göre trizomi 21 için kombine riski <1: 1500 ve > 1: 1500 olan hasta sayısı ve P değerleri.

	<1:1500	>1:1500	P values with reported risk	P values
Reported combined trisomy 21 risk	58	486	-	
Combined trisomy 21 risk calculated worst-case scenarios with AACB MU [a]	94	450	<0.001	0.06 [c]
Combined trisomy 21 risk calculated worst-case scenarios with Nordtest MU [b]	99	445	<0.001	
Combined trisomy 21 risk calculated best-case scenarios with AACB MU [a]	38	506	<0.001	0.5 [d]
Combined trisomy 21 risk calculated best-case scenarios with Nordtest MU [b]	36	508	<0.001	

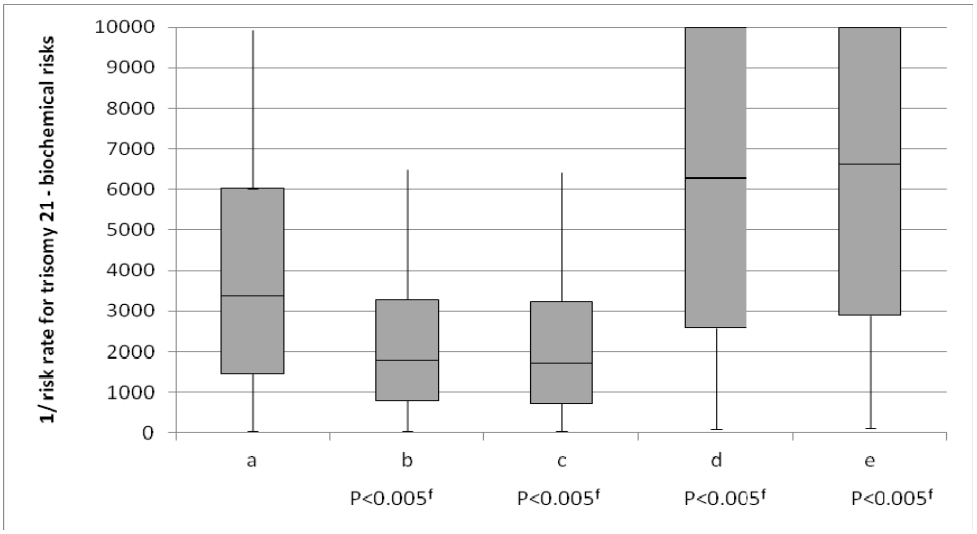
[a]: Measuremet uncertainty calculated with Australasian Association of Clinical Biochemists guideline
[b]: Measuremet uncertainty calculated with Nordtest guideline
[c]: P value between worst-case scenarios calculated with AACB MU and Nordtest MU
[d]: P value between best -case scenarios calculated with AACB MU and Nordtest MU



Graphic 1. Box plot graphic for trisomy 21 biochemical risks of best and worst-case scenarios calculated with AACB and Nordtest guidelines

Grafik 1: Trizomi 21 biyokimyasal riski için AACB ve Nordtest kılavuzları ile hesaplanan en iyi ve en kötü durum senaryolarının box-plot grafiği

a: Reported biochemical trisomy21 risk
b: Biochemical trisomy 21 risk calculated worst-case scenarios with Australasian Association of Clinical Biochemists guideline
c: Biochemical trisomy 21 risk calculated worst-case scenarios with Nordtest guideline
d: Biochemical trisomy 21 risk calculated best-case scenarios with Australasian Association of Clinical Biochemists guideline
e: Biochemical trisomy 21 risk calculated best-case scenarios with Nordtest guideline
f: P values with reported risk



Graphic 2. Reported and recalculated results of 211 patients reported other than <1:10000 and >1:50

Grafik 2: <1:10000 ve >1:50 haricinde raporlanan 211 hastanın raporlanan ve yeniden hesaplanan sonuçları

a: Reported biochemical trisomy21 risk
b: Biochemical trisomy 21 risk calculated worst-case scenarios with Australasian Association of Clinical Biochemists guideline
c: Biochemical trisomy 21 risk calculated worst-case scenarios with Nordtest guideline
d: Biochemical trisomy 21 risk calculated best-case scenarios with Australasian Association of Clinical Biochemists guideline
e: Biochemical trisomy 21 risk calculated best-case scenarios with Nordtest guideline
f: P values with reported risk

For trisomy 13/18 469 of 544 patients have rate as <1:10000. So median values of reported and all the other scenarios' risk rates are <1:10000. But both of the worst-case scenarios with AACB and Nordtest guidelines have changed 75 patients results. 62 patients results were changed by both of the best-case scenarios with AACB and Nordtest guidelines. And these changes were statistically significant (P<0.005).

DISCUSSION

The clearest indicators of the effect of measurement uncertainty on results is significant change in the number of patients requiring further investigation for trisomy 21. Excluding the results of <1:10000 or >1:50, median of the risk rate doubled in the worst scenarios and halved in the best scenarios. Moreover different sensitivity and specificity rates in the literature related to first trimester prenatal screening may be due to different measurement uncertainties.

It is seen that the result of the uncertainties calculated according to the Nordtest guideline of the medium level of PAPP-A are higher than that calculated according to the AACB guideline. This difference is due to the fact that RMSbias is higher than expected. There was a bias result 18% in sample 8, which has the target value close to the medium level. In that EQC report, the Z score (SDI) was reported as 1.81 due to the breadth of the distribution (CV = 9.2%). Low level of PAPP-A is related to the risk more than medium and high levels. This explains why there is no significant difference between the number of patients classified as risky, while there is a significant difference between the medians of the risk rates with different guidelines.

Reporting uncertainty with the result may be perceived as an error by the clinician and may cause distrust to the result. However, the physicians should be aware that each result contains uncertainty. And they should take this into account when deciding risky transactions such as CVS. The lowest and the greatest risks can be given during the reporting of first trimester prenatal screening results with uncertainty of measurement. For example, the patient's combined risk of trisomy 21 may be reported as 'between 1:1180 - 1:4470' instead of 1: 2260. This may be confusing because results are on the different sides of 1:1500 cut-off but the physician should be aware of this.

Calculating measurement uncertainty at different levels is more plausible than to calculate a single measurement uncertainty. But it can be distractive. For example, in our study, low level of PAPP-A was 1.89 IU/L and medium level was 9.11 IU/L. It is unclear that the value in the middle of these two values (ie 5.5 IU/L) will be included on which side. In our study, we considered a patient's PAPP-A result of 5.49 IU/L as low level. We performed the transactions with 11.21% and we found the minimum and maximum possible values as 4.87-6.11 IU/L. Another patient's PAPP-A result was 5.53 IU/L, which was closer to the medium level. We made transactions with 23.01%. And we found the minimum and maximum values as 4.26-6.80 IU/L. The difference between the possible outcomes of first trimester prenatal screening has changed considerably due to the change in the category of these two results, with a difference of only 0.04 IU/L. As a solution, if there is too much difference between the measurement uncertainty rates of two different levels and this is due to a single bias value, as in our study, a single measurement uncertainty value can be calculated for all levels. However, if the bias of a certain level is found to be consistently high, we do not recommend doing so because it can be said that the performance is not very good at that level.

Although there are opposite views we believe precision only is not sufficient to determine measurement uncertainty. The bias of the results should also be used in the uncertainty calculation because good precision of the result does not give information about the accuracy. Also, ISO Technical Specifications 20914 does not recommend ignoring bias

completely. Using of bias and imprecision in a pythagorean equation reduces the effect of both compared to linear addition. Therefore, although AACB can be applied more easily we recommend the Nordtest guideline. For more realistic results of RMSbias, we recommend to use as many EQC results as possible, so it can be preferred to use more frequently used EQC programs.

Although the ultrasound measurement procedure is standardized, NT measurement includes uncertainty. But we couldn't include it and this is one of the limitations of our study. If the uncertainty of NT is included in the study in addition to the uncertainties of biochemical constituents, we think that the range will be widened. Another limitation is the number of patients. Besides, the extended measurement uncertainty (coverage factor 2) covers a confidence interval of 95%. The probability of the 2.5th centile PAPP-A aligning with the 97.5th centile free β-hCG to give a worst case scenario is relatively low. The same is true for the occurrence of the best case scenario and this may lead to a overestimated effect of measurement uncertainty on screening performance. Also, if the exact values of the results <1:10000 and >1:50 were known, more reliable results could be obtained in statistics.

The uncertainty of measurement can be used as a quality indicator and gives the clinician more detailed information about the results. Measurement uncertainty becomes more important in tests which are calculated by using of multiple parameters. Increasing the number of studies will raise awareness on the subject and it will contribute to the creation of target values for measurement uncertainty.

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