COMBINED FIRST TRIMESTER PRENATAL SCREENING PROGRAM AT DANIEL DE MATOS MATERNITY

Ana Torgal[1], Sofia Franco[2], José Barros[1,2]

[1] Faculty of Medicine, University of Coimbra, Portugal

[2] Daniel de Matos Maternity, Coimbra, Portugal

Corresponding author:

Ana Torgal

E-mail address: anatorgal@gmail.com

ABSTRACT

Introduction:

Screening for trisomy 21 (T21) by a combination of fetal nuchal translucency (NT), maternal serum free-β-human chorionic gonadotrophin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) is now part of routine care in the first trimester of pregnancy.

The objective of this study was to retrospectively review data from first trimester combined screening program for T21 performed in Daniel de Matos Maternity (MDM) between 2011 and 2013, critically assessing the following parameters: median age; detection rate; false positive rate; biochemical markers and NT distribution.

Methods:

Retrospective review of first trimester T21 screening at MDM between 2011 and 2013.

Only singleton pregnancies for which trisomy 21 status (affected or not affected) was known (ascertained through cytogenetic study or at birth) were included.

Screening parameters (maternal age, NT, PAPP-A and β-hCG) for affected and unaffected pregnancies were assessed and compared. Screening test performance indicators (detection rate, false positive rate, and positive predictive value) were calculated and critically evaluated.

Results:

There were 3537 women with singleton pregnancies with known outcomes. 179 cases (4.82% of the original sample) were lost for follow-up. 2750 women (77.75%) were younger than 35, whilst 787 (22.25%) were 35 or older.

There were 66 pregnancies (1.87%) with positive screening tests, 1.24% in women aged less than 35 and 4.07% of women aged 35 or older. Overall detection rate was 71.43% (5/7) and false

positive (FP) rate was 1.73% (1.09% in women aged less than 35 and 3.95% of women aged 35 or older). The positive predictive value of the screening test was 1:13.

However diverging from theoretical values, NT, β-hCG and PAPP-A distribution generally follow the normal curve. As expected, differences in these parameters between affected and unaffected groups showed statistically significance.

Discussion and Conclusion:

Effective screening for trisomy 21 was achieved in the first trimester of pregnancy with a detection rate of 71.4% and a false-positive rate of less than 2%, but conclusions should however be withdrawn with caution, due to the effect of small numbers. Performance improvement could be achieved by adjusting individual parameters medians. Risk cut-off variations could improve detection rate, still leaving the false positive rate at acceptable levels. Further cases are needed in order to continuously evaluate screening performance.

Keywords: pregnancy, prenatal diagnosis, first-trimester screening; trisomy 21; aneuploidies; nuchal translucency; serum PAPP-A; serum free β –hCG.

RESUMO

Introdução:

O rastreio de trissomia 21 (T21) através da combinação da medida da translucência da nuca (TN) e doseamento da gonadotrofina coriónica humana fracção beta (β-hCG livre) e proteína plasmática associada à gravidez (PAPP-A) faz parte do acompanhamento obstétrico de rotina no primeiro trimestre da gravidez.

Este estudo teve como objectivo analisar retrospectivamente os dados referentes ao rastreio combinado do primeiro trimestre de T21 realizado na Maternidade Daniel de Matos (MDM) entre 2011 e 2013, avaliando criticamente os seguintes parâmetros: idade mediana da grávida; taxa de detecção (TD); taxa de falsos positivos (FP); distribuição dos marcadores bioquímicos e TN.

Métodos:

Análise retrospectiva do rastreio do primeiro trimestre da T21 na MDM entre 2011 e 2013.

Foram incluídas apenas gestações de feto único sobre as quais se dispunha de informação relativa à existência ou ausência de T21 (confirmada por estudo citogenético ou ao nascimento).

Compararam-se os parâmetros de rastreio (idade materna, TN, PAPP-A e β-hCG) em gestações afectadas e não afectadas, tendo sido avaliados os indicadores de desempenho do rastreio (TD, FP, valor preditivo positivo - VPP).

Resultados:

Foi obtida informação sobre 3537 gestações de feto único. Não existiam dados completos sobre a evolução da gravidez em 179 casos (4,82%). 2750 grávidas (77,75%) tinham menos que 35 anos, enquanto que 787 (22,25%) tinham idade igual ou superior a 35.

66 gestações (1,87%) tiveram testes de rastreio positivos, dos quais 1,24% correspondiam a mulheres com idade inferior a 35 anos e 4,07% a mulheres acima dessa idade. A TD global foi de

71,43% (5 em 7), com 1,73% de FP (1,09% em mulheres com idade inferior a 35 anos e 3,95% em mulheres com idade igual ou superior a 35). O VPP do teste de rastreio foi de 1:13.

Verificou-se que os parâmetros de rastreio TN, β-hCG e PAPP-A, embora divirjam dos valores teóricos, seguem uma distribuição normal, existindo, diferenças estatisticamente significativas entre os grupos de gestações afectadas e não afectadas.

Discussão e Conclusões:

O teste de rastreio apresentou uma TD de 71,4% e taxa de FP inferior a 2%, embora as conclusões devam ser encaradas com prudência, dada a dimensão da amostra. A performance do rastreio pode ser melhorada ajustando as medianas dos parâmetros de rastreio. Variações no *cut-off* do teste de rastreio podem permitir aumentar a TD, mantendo-se a taxa de FP em valores aceitáveis. Será necessário continuar a avaliar o desempenho do teste com maior número de casos.

Palavras-chave: gravidez, diagnóstico pré-natal, rastreio do 1º trimestre; trissomia 21; aneuploidias; translucência da nuca; proteína plasmática associada à gravidez (PAPP-A); gonadotrofina coriónica humana fracção beta (β-hCG livre).

INTRODUCTION

With thousands of women presenting for prenatal care in Portugal annually and current recommendations to offer trisomy 21 (T21) screening/testing to all [1], there is no doubt that the quality of prenatal screening programs has an important impact on the well-being of women and children, besides all social and economic implications. In fact, aneuploidies are major causes of perinatal death, childhood handicap and a significant burden for healthcare systems [2].

Trisomies 13, 18 and 21 are practically the only nonmosaic autosomal trisomies detected at amniocentesis or chorionic villus sampling (CVS), since virtually all others miscarry before. Figures for T21 are of most interest, as this condition produces a major mental handicap, implies a significant burden for parents, and is the most common single chromosome defect in newborns. These full aneuploidies are in the majority of cases the result of meiotic nondisjunction, which can happen at any maternal age, but more frequently in older mothers [3].

The detection of chromosomal disorders and other fetal abnormalities constitutes the most frequent indication for invasive prenatal diagnosis (PND). However, invasive testing, by amniocentesis or CVS, is associated with a risk of miscarriage [2]. Amniocentesis was initially offered only to women with a minimum age of 40 years. Gradually, as the application of amniocentesis (performed around 16 weeks of pregnancy) became more widespread and it appeared to be 'safe', the 'high-risk' group was redefined to include women with a minimum age of 35 years; this group constituted at that time approximately 5% of the pregnant population [4].

The age of pregnant women has increased in developed countries and in Portugal, according to recent data (2013), this screen-positive group now constitutes about 26.3% of all pregnancies [5]. Whilst in the 1970s the main method of screening for aneuploidies was by maternal age, in the

1990s the emphasis shifted to first trimester screening, when it was realized that the great majority of fetuses with major aneuploidies can be identified by a combination of maternal age, fetal nuchal translucency (NT) thickness (optimal measurement between 11 and 13 weeks and 6 days), maternal serum free β-human chorionic gonadotrophin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) levels [2, 6].

In T21, decreased concentration of PAPP-A and increased β-hCG are observed. These parameters greatly depend on gestational age and are therefore expressed as gestational age-adjusted multiples of the median (MoM) for risk calculation [7]. Gaussian distributions of log10 (MoM) in T21 and unaffected pregnancies are then derived, and the ratio of the heights of distributions at a particular MoM, which is the likelihood ratio for T21, is used to modify the *a priori* maternal age-related risk to derive patient-specific risk [2]. Although ideally each center should establish its own medians, this process is laborious, costly, and time-consuming [7], requiring a large number of cases. Therefore, several commercial software are available to calculate individual risk, using gestational default medians for these parameters.

Several prospective studies in hundreds of thousands of pregnancies have demonstrated that the risk of T21 increases with both maternal age and fetal NT thickness and that in pregnancies with low fetal NT the maternal age-related risk is decreased [2, 8].

These ultrasonographic and biomarkers can be combined to provide more effective screening than either method individually [2].

Several studies have demonstrated that for a 5% false-positive rate, the first-trimester combined test identifies more than 80% of T21 pregnancies [2, 6].

The objective of this study was to retrospectively review data from first trimester combined screening program for T21 performed in Daniel de Matos Maternity (MDM) between 2011-2013,

critically assessing: median age; detection rate; false positive rate; biomarkers and NT distribution.

METHODS

Screening for T21 at MDM is carried out by first-trimester combined test at 11 to 13 weeks and 6 days of gestation.

The Brahms Kryptor system (Thermo Scientific, Germany) was used to measure PAPP-A and free β-hCG.

Gestational age was determined by the Astraia software (Munich, Germany), using the crown-rump length (CRL), which should range from 45 to 84 mm. NT thickness was measured only when the CRL was at least 45 mm.

The Astraia software was used for the routine risk calculation. A woman was classified as screen-positive if her risk estimate for T21 was equal to or greater than 1 in 300, and routinely offered a definitive diagnosis by CVS or amniocentesis for fetal karyotyping.

Maternal demographic characteristics, ultrasonographic measurements, biochemical results, calculated risks, karyotype results and pregnancy outcomes were extracted from the MDM registries and include information from multiple sources (PND consultation database; deliveries database; neonatology unit records).

A search of the databases was done to identify all singleton pregnancies in which first trimester screening by fetal NT, PAPP-A and free β-hCG was carried out from 1 January 2011 and 31 December 2013. From these, only cases for which trisomy 21 status (affected or not affected) was known (ascertained through cytogenetic study or at birth) were included.

Cytogenetic results were described according to the International System for Chromosome Nomenclature, 2013 [9]. Common chromosomal variants that are not clinically significant (e.g. pericentric inversion of chromosome 9) were classified as normal.

Statistical analysis was performed using Stata software (College Station, USA). $P < 0.05$ was taken as the formal level of statistical significance. The chi-square test was used to evaluate differences between age groups. Fisher's exact test was used for reduced size samples (less than 5 observations for a given parameter).

Ethical approval for this study was obtained from the Ethics Commission of the Faculty of Medicine of the University of Coimbra.

RESULTS

3716 women participated in the screening program, of which 179 (4.82%) were lost for follow-up (including fetal losses for which karyotype was not available). The remaining 3537 pregnancies with a known outcome constitute our population of study.

Average age of women at first ultrasound was 31.3 (standard deviation of 4.6) and the median age was 31.4, the oldest being 46.8 years old. 2750 women (77.75%) were younger than 35, whilst 787 (22.25%) were 35 or older.

There were 3505 deliveries at MDM (94.32% of screening program participants, and 99.10% among our population of study).

66 pregnancies (1.87% of the study population) had a positive screening test, of which 5 had trisomy 21 (Table 1). Analyzing by age, 1.24% of all women aged less than 35 and 4.07% of women aged 35 or older had positive screening tests. Differences between age groups were statistically significant ($p < 0.05$).

	N	Unaffected[1]	T21	Other[3]
Positive screen T21 (1:300) – overall	66	59[2]	5	2
Positive screen T21 (1:300) – <35yrs	34*	29	4	1
Positive screen T21 (1:300) – ≥35yrs	32*	30[2]	1	1

Table 1 – Pregnancies with positive screening tests. *p<0.05 (chi-square test)

[1] Pregnancies without T21 fetuses or other known chromosomal abnormality; [2] Includes two cases for which karyotype was not available, but with apparently unaffected newborns (one PND refusal and one case due to culture failure); [3] Abnormal karyotypes other than T21.

Although there were only 66 positive screening tests, cytogenetic studies were performed on a total of 247 fetuses (183 pregnancies with negative screening tests). Reasons for referral included abnormal ultrasound scan (mainly in the second trimester), previous chromosome anomaly, carrier of a structural rearrangement, or maternal infection. Table 2 depicts the types of fetal chromosome abnormality detected.

Karyotypes	N
46,XX	111
46,XY	118
T13	2
T18	2
T21[1]	7
Others[2]	7
Total	247

Table 2 – Karyotype results in screen positive and screen negative pregnancies.

[1]Including 1 mosaic 47,XX,+21/46,XX.nuc ish(D21S259,D21S341,D21S342)x3[188/200];

[2]Including: 45,XY,der(13;14)(q10;q10); 46,XX,del(15)p11.2; 46,XX/45X; 46,XX/47XX,+mar; 46,XY,t(11;18)p(15.1;q23)dn; 46,XY/46,XY,+t(2,6)(q24~31;q25~26)dn; 47,XXY.

Table 3 describes the characteristics of the trisomic and unaffected groups, showing statistically significant differences in NT, β-hCG and PAPP-A parameters. Table 4 contains more detailed information on the T21 cases.

Characteristic	Unaffected (n=3530)	Trisomy 21 (n=7)	*p*
Maternal age, years	31.0 (28.0-34.0)	34.0 (32.5-37.5)	0.112 (NS)
Crown-rump length, mm	63.8 (57.8-70.0)	69.3 (66.0-75.3)	0.075 (NS)
NT, mm	1.4 (1.2-1.7)	2.3 (1.9-3.3)	<0.001*
Serum PAPP-A, MoM	1.094 (0.771-1.507)	0.555 (0.524-1.273)	0.025*
Serum free β-hCG, MoM	0.819 (0.563-1.020)	2.320 (1.867-2.943)	0.002*

Table 3 – Characteristics of the study population. Data are presented as median (Inter Quartile Range). *Differences between groups are statistically significant ($p<0.05$). NS – Non significant.

Case	Maternal age	CRL (mm)	NT (mm)	PAPP-A (MoM)	β-hCG (MoM)	T21 screening risk	Karyotype result
1	31	69.7	4.7	0.49	1.33	1:4	47,XX,+21
2	31	75.1	2.6	1.12	2.32	1:24	47,XY,+21
3	37	67	2.3	0.57	2.05	1:47	47,XX,+21/46,XX.nuc ish(D21S259,D21S341, D21S342)x3[188/200]
4	34	69.3	1.7	0.56	3.15	1:80	47,XY,+21
5	34	63	1.7	0.54	2.62	1:129	47,XY,+21
6	29	63	2.8	0.45	0.50	1:382	47,XX,+21
7	38	75.7	2	1.72	2.87	1:556	47,XX,+21

Table 4 – Clinical information in T21 cases

As it is implicit from Tables 1 and 2, there were two False Negative (FN) screening tests, with an overall detection rate (DR) of 71.43% (Table 5). DR differences between age groups were not statistically significant.

	True positive cases (TP)	False Negative cases (FN)	DR
T21 Overall	5	2	71.43% (5/7)
T21 <35	4	1	80.00% (4/5)*
T21 ≥35	1	1	50.00% (1/2)*

Table 5 – Detection rate. *Non-significant – Fisher's exact test statistic value is 0.52381.

False positive (FP) rate was below 5% overall and in both age groups (Table 6). Differences between age groups were statistically significant ($p<0.05$).

	FP	FP rate
T21 Overall	61	1.73%
T21 <35	30	1.09%*
T21 ≥35	31	3.95%*

Table 6 – FP rate. *$p<0.05$ (chi-square test)

Positive predictive value (PPV), expressed as the odds ratio of the number affected to the number unaffected among those pregnant women considered "high risk" for T21, was 1:13 (Table 7), without statistically significant differences between age groups.

	TP cases	FP cases	PPV
T21 Overall	5	61	7.58% (1:13)
T21 <35	4	30	11.76% (1:9)*
T21 ≥35	1	31	3.13% (1:32)*

Table 7 – Positive predictive value of the screening test. *Non-significant – p=0.1849 (chi-square test).

The distribution of the screening parameters was also assessed. Figure 1 shows the distribution of fetal nuchal translucency thickness by crown-rump length. The median and the regressed 5th and 95th centile lines are also shown.

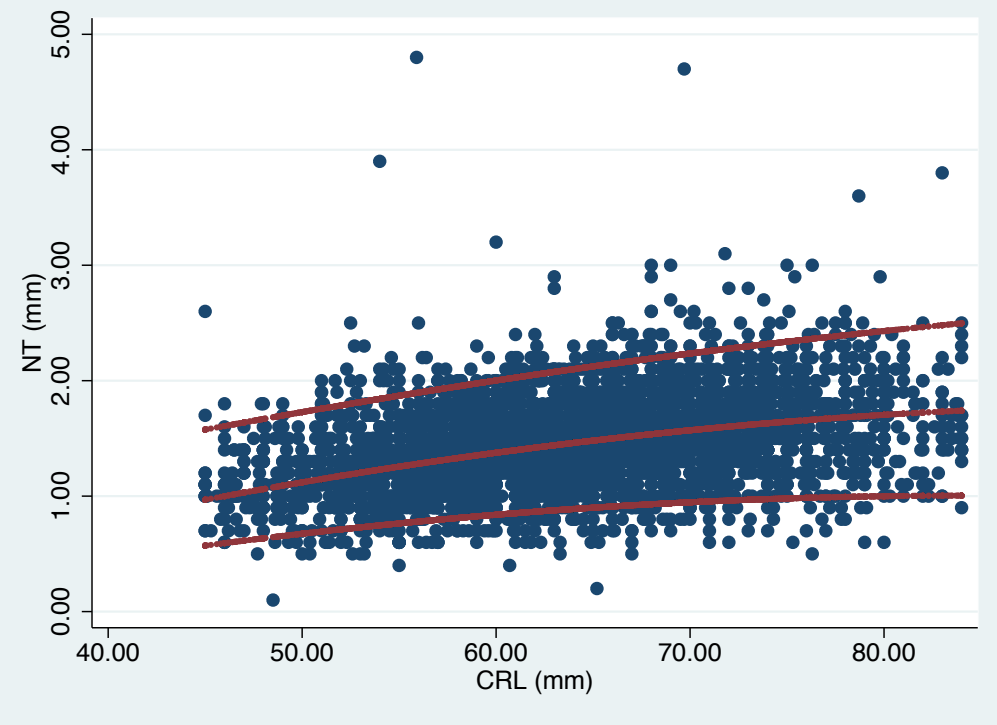

Figure 1 - Distribution of fetal nuchal translucency thickness by crown-rump length. Red lines: median and regressed percentiles 5 and 95 lines. *NT – Nuchal Translucency; CRL – Crown-rump Length.*

NT distribution around the median generically follows the normal distribution, as can be seen in Figure 2. The deviance from the median also follows normal distribution, as shown in Figure 3.

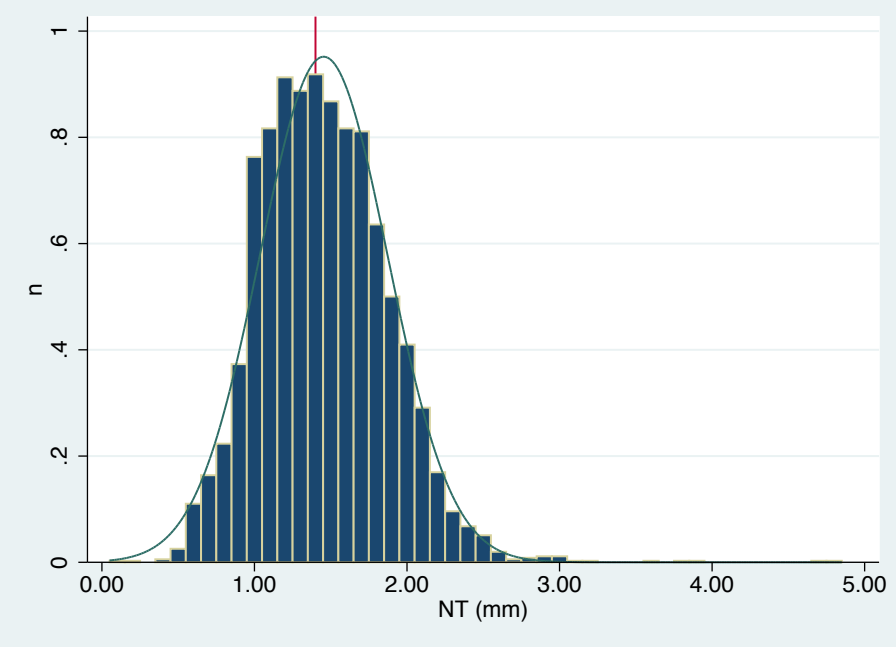

Figure 2 – NT distribution by number of cases.

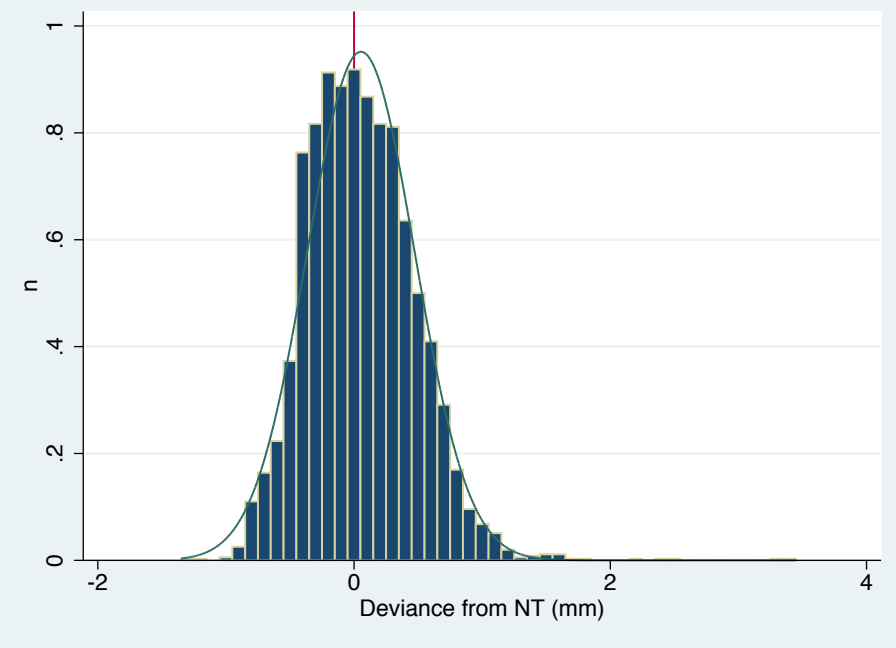

Figure 3 – NT deviance from the median.

Figure 4 shows the NT deviation of from the Astraia software theoretical median.

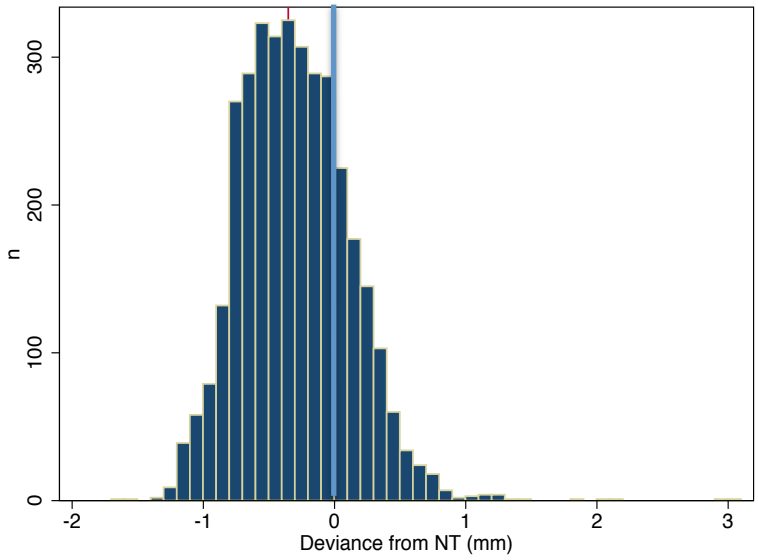

Figure 4 – NT deviance from the Astraia software theoretical median [10], calculated as 10^(-0.8951 + 0.02941 * CRL - 0.0001812 * CRL^2), where the CRL variable is the median CRL from our population.

Biochemical markers' distribution was also evaluated. All measured β-hCG and PAPP-A results were expressed in MoM values for the appropriate gestational date and transformed to log10 values. Figures 5 and 6 show the distributions (both following the normal curve) of log10Beta and log10PAPP-A.

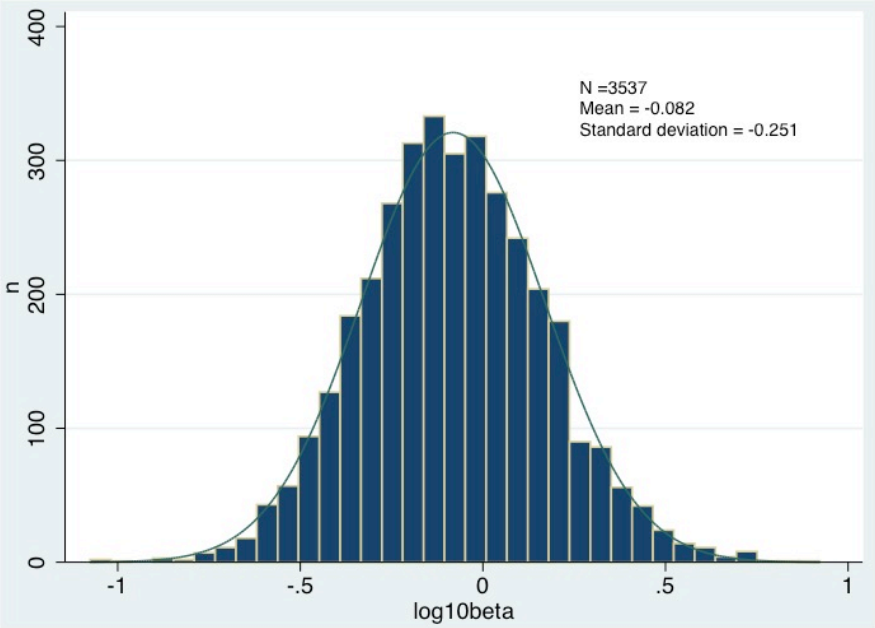

Figure 5 – Log10Beta distribution.

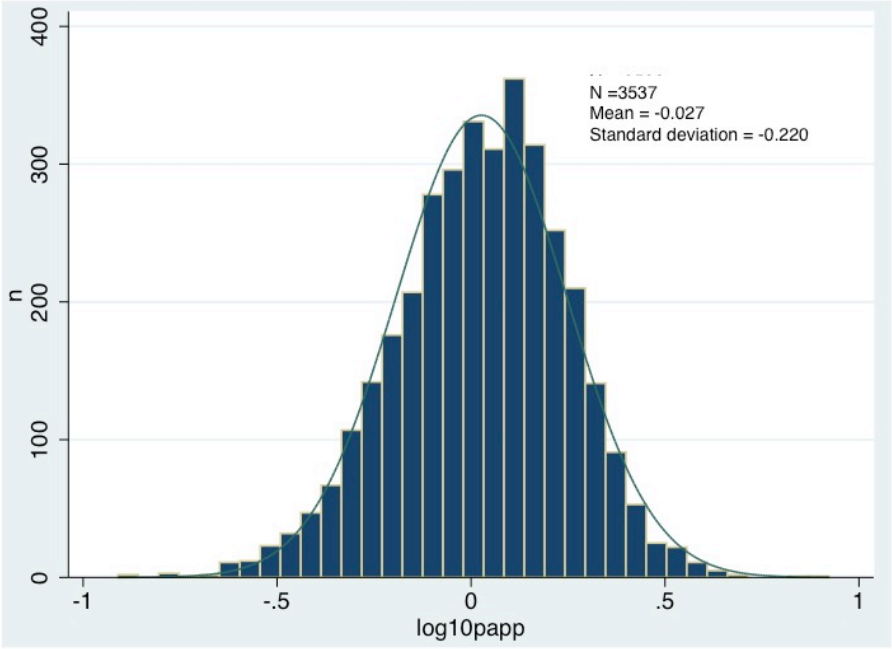

Figure 6 – Log10PAPP-A distribution.

DISCUSSION

In many developed countries prenatal screening and diagnosis of T21 is now a routine part of care in pregnancy.

Assessing the effectiveness of prenatal screening is not straightforward and there are inevitably some limitations in interpretation of the data presented here.

There were 179 cases lost for follow-up (4.82% of all women participating in the screening program), so conclusions outlined from this study should be interpreted with caution. In fact, it is impossible to know if the population lost for follow-up has a proportional incidence of trisomy 21 as those who remained in the study, namely because being this a retrospective observational study, the effect of spontaneous abortions in undetected T21 pregnancies was obviously not taken into account (affected pregnancies are more likely to miscarry than those unaffected [2, 6]).

In our study population there were 7 cases of confirmed T21, 5 of which from women younger than 35. This points to an overall prevalence of 1:505 (19.8 in 10,000) and to a prevalence of 1:550 below 35 years and 1:393 above or at this age.

Based on data from national registries of births from England and Wales in 2011, the estimated prevalence of trisomy 21 at 12.5 weeks of gestation (20% of women aged 35 or older) was calculated as 1:340 (29.4 in 10,000) [11, 12, 13]. This could be used as a reference to our study, since our population is relatively similar (the proportion of women aged 35 or older was 22.25%) and we calculate the risk at the screening point (median CRL corresponding to 12 weeks and 5 days). The difference of 1 in 1000 (9.6 in 10,000) between our population of study and the above mentioned estimated risk [12], could either be due to the effect of small numbers or to the loss of cases for follow-up.

The 22.25% proportion of women aged 35 or older stands if favor of doing the screening process based in criteria other than age alone. If amniocentesis were to be performed in all women considered at "high risk" for fetal aneuploidies solely on the basis of their age (thus assuming a theoretical 100% uptake of this procedure by women aged 35 years-old or above), 787 amniocentesis would have been done, which would have allowed us to identify only 2 of the 7 T21 cases (corresponding to a 28.57% DR). Besides, considering a 1.0-2.0% miscarriage rate associated to the CVS or amniocentesis procedures [14], 8 to 16 pregnancies could be lost.

As expected and depicted in Table 1, the probability of having a positive screening test increases with age and differences between groups were statistically significant.

There were statistically significant differences between affected and unaffected pregnancies in NT, PAPP-A and β-hCG but not in population demographics (maternal age and gestational age).

The 1.09 median of the PAPP-A MoM for unaffected pregnancies, which is the same as the overall population's median, is 9% above the theoretical value of 1.0 for the median of MoMs. Accordingly, the 0.82 median of the β-hCG MoM for unaffected pregnancies (also the same as the overall population's) means that this parameter is being underestimated by nearly 18%. This means that β-hCG, more meaningfully, is being underestimated, but also PAPP-A is overestimated, and need to be adjusted in order to avoid losing cases, since T21 pregnancies are associated with high β-hCG and low PAPP-A levels. These differences were statistically significant ($p<0.05$).

The screening test's DR was 71.43% overall, being 80.0% below 35 years old and only 50.0% above that age (differences without statistical significance). The overall FP rate was 1.73% (1.09% below 35, 3.95% above the age of 35, statistically significant). These values are lower than the percentages usually reported, with DR of at least 80.0% for a FP rate of 5% [2, 6, 15]. In

the SURUSS trial (a large-scale trial based on 47,053 singleton pregnancies) [6] a FP rate of 1.5% was associated with a DR of 70% (while considering maternal age, NT, PAPP-A and β-hCG as screening parameters), which is in line with our overall results. However, since we're dealing with small numbers, it is important to stress that there's always a random effect (which seems more noticeable especially above the age of 35, with only two T21 cases) and so conclusions should be taken with caution.

For continuous variables the choice of a cut-off level that determines whether a value is positive or negative is arbitrary as there is no intrinsic division between the distributions of affected and unaffected pregnancies. So, the choice will be influenced by the perceived relative importance of three factors: DR, FP rate, and the PPV. Since there is a trade-off between DR and FP rate, one possibility to increase the DR would be to set the cut-off for positive screen test at 1:400. This would increase the DR to 85.7% (due to the effect of small numbers, since only one of the FN cases would became positive), leaving the FP rate at 2.52%.

It is also necessary to clarify that in this study we are considering the risk for trisomy 21 at the screening point, not the risk at birth (which, due to the possibility of fetal losses, is lower). The conversion from one risk to another is normally made by multiplying the risk at the screening point by 1/0.77, to allow for the general fetal loss of Down's syndrome pregnancies from this time in pregnancy until term [6].

The 2 false negative (FN) cases included one borderline screening test (with a risk estimation of 1:382) and another case in which the calculated risk was 1:586. In both cases, little variations in NT measurements and/or biochemical markers levels could turn the tests positive. Both FN cases ended with medical termination of pregnancy. In case 6 (Table 4), T21 was diagnosed by amniocentesis at 16 weeks. The patient was referred for invasive procedure due to altered

ultrasound markers (NT above percentile 95 and reversed ductus venosus). Case 7 (Table 4) had a normal ultrasound, with NT below percentile 95, and chose to perform amniocentesis due to maternal anxiety.

The PPV of the screening test was 1:13 overall and lower (1:32) for women older than 35, without statistically significant differences between age groups.

Despite the potential predictive value of nuchal translucency [8, 16], consistent and accurate NT measurements can be difficult to obtain and NT has proven to be extremely operator-dependent [17, 18]. Figure 1 shows that the distribution of NT measurements by CRL is well between percentiles 5 and 95, as defined by the Fetal Medicine Foundation (London, United Kingdom) criteria, and so gestational age doesn't seem to lead neither to overestimations or underestimations of this parameter [4]. NT absolute values distribution generally follows the normal curve, as can be seen in Figure 2. Figure 3 shows that the deviance form the NT median curve follows the normal distribution, which is a sign of consistency, since it is well established that larger deviations in median NT have a substantial adverse effect on the performance of screening [19]. It is impossible however to compare the distribution of affected and unaffected pregnancies in a statistically significant way, given the low number of affected pregnancies (only 7).

Figure 4 shows that NT determinations are generally below the Astraia software theoretical median, which can lead to an underestimation of T21 cases.

It is well established in the literature that adopting sonographer-specific medians (in our study we only have overall measurements) allows for systematic differences between sonographers and will ensure that sonographers acquire sufficient initial experience and continuing experience to derive reliable values for NT [6]. Also, NT medians derived from individual sonographers have

been shown to result in higher detection rates and lower false positive rates than using either center- or population-based medians [20].

CONCLUSIONS

Effective screening for trisomy 21 was achieved in the first trimester of pregnancy with a detection rate of 71.4% and a false-positive rate of less than 2%. Adjustments in risk cut-off could improve detection rate, still leaving the false positive rate at acceptable levels. The effect of small numbers is however present and could lead to data misinterpretations.

Screening programs should be continuously evaluated and adjusted until an appropriate number of cases are achieved. So, further monitoring is needed to get consistent results from a larger population and to assess differences between affected and unaffected pregnancies.

Even though screening parameters distribution showed consistency, the adoption of well-described measures to optimise screening capacity, like individual monitoring of NT measurements, median adjustments and careful internal and external quality control programs for laboratorial parameters' determinations are preventive measures that could certainly optimise the overall screening performance.

AGRADECIMENTOS

Ao Professor Doutor José Barros e à Dra. Sofia Franco, pela orientação, disponibilidade, compreensão e sábios conselhos.

À Dra. Júlia Cardoso pela ajuda no processo de recolha de dados, sem a qual este trabalho seria impossível.

À Professora Doutora Joana Bastos pela enormíssima ajuda no tratamento estatístico dos dados.

BIBLIOGRAPHY

1. Circular Normativa nº6/DSMIA, 07.07.1997. Direcção-Geral da Saúde 1997;
2. Nicolaides KH. Screening for fetal aneuploidies at 11 to 13 weeks. Prenat Diagn 2011; 31: 7–15;
3. Gardner RJM, Sutherland GR. Chromosome abnormalities and genetic counselling. 2004; 3rd Edition, Oxford University Press, New York;
4. Nicolaides KH. The 11-13+6 weeks scan. Fetal Medicine Foundation 2004; http://www.fetalmedicine.com/synced/fmf/FMF-English.pdf (accessed at 31.01.2015);
5. Instituto Nacional de Estatística. Nados-vivos (N.º) por Local de residência da mãe, Grupo etário da mãe e Condição perante o trabalho da mãe; Anual. http://www.ine.pt/xportal/xmain?xpid=INE&xpgid=ine_indicadores&indOcorrCod=0005954&contexto=bd&selTab=tab2 (accessed at 03.02.2015);
6. Wald NJ, Rodeck C, Hackshaw AK,Walters J, Chitty L, Mackinson AM. First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS). Health Technol Assess 2003;7(11);
7. Sørensen S, Momsen G, Sundberg K, Friis-Hansen L, Jørgensen FS. Clinical Chemistry 2011; 57:7:1023-1031;
8. Nicolaides KH. Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. Am J Obstet Gynecol 2004; 191: 45–67;
9. International Standing Committee on Human Cytogenetic Nomenclature. ISCN 2013: an international system for human cytogenetic nomenclature 2013. Editors Lisa G Shaffer, Jean McGowan-Jordan, Michael Schmid, Basel, Switzerland;

10. Wright D, Kagan KO, Molina FS, Gazzoni A, Nicolaides KH. A mixture model of nuchal translucency thickness in screening for chromosomal defects. Ultrasound Obstet Gynecol 2008; 31: 376–383;
11. Wright DE, Bray I. Estimating birth prevalence of Down's syndrome. J Epidemiol Biostat 2000; 5: 89 – 97;
12. Nicolaides KH, Wright D, Poon LC, Syngelaki A, Gil MM. First-trimester contingent screening for trisomy 21 by biomarkers and maternal blood cell-free DNA testing. Ultrasound Obstet Gynecol 2013; 42: 41–50;
13. Springett A, Budd J, Draper ES, Fitzsimons K, Kurinczuk J, Rankin J, Rounding C, Stoianova S, Tonks A, Tucker D, Wellesley D, Wreyford B, Morris JK. Congenital Anomaly Statistics 2011: England and Wales. London: British Isles Network of Congenital Anomaly Registers. 2013;
14. Tabor, A., Vestergaard, C. H. F. and Lidegaard, Ø. Fetal loss rate after chorionic villus sampling and amniocentesis: an 11-year national registry study. Ultrasound Obstet Gynecol, 2009; 34: 19–24
15. Morris JK, Mutton DE, Alberman E. Revised estimates of maternal age specific live birth prevalence of Down's syndrome in the absence of antenatal screening and selective termination. J Med Screen 2002; 9:2–6;
16. Nicolaides KH, Spencer K, Avgidou K, Faiola S, Falcon O. Multicenter study of first-trimester screening for trisomy 21 in 75 821 pregnancies: results and estimation of the potential impact of individual risk-orientated two-stage first-trimester screening. Ultrasound Obstet Gynecol 2005; 25: 221–226.
17. Malone FD, D'Alton ME: First trimester sonographic screening for Down syndrome. Obstet Gynecol 2003. 102:1066-1079;

18. Fuchs KM, Peipert JF. First Trimester Down Syndrome Screening: Public Health Implications. Sem Peri. 2005. 267-271;
19. Kagan KO, Wright D, Etchegaray A, ZhouY, Nicolaides KH. Effect of deviation of nuchal translucency measurements on the performance of screening for trisomy 21. Ultrasound Obstet Gynecol 2009; 33: 657–664;
20. Malone FD, Wald NJ, Canick JA. Use of overall population, center-specific, and sonographer-specific nuchal translucency medians in Down syndrome screening: which is best? Am J Obstet Gynecol 2003.189:S232.