

MESTRADO INTEGRADO
MEDICINA

Budget Impact Model – Harmony Prenatal Test

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2023



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Dissertação de Mestrado Integrado em Medicina

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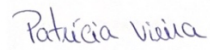
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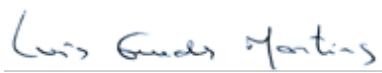
Porto, maio, 2023

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Agradecimentos

Início por agradecer a todos os que contribuíram para o meu percurso académico ao longo destes seis anos.

Ao meu orientador, Prof. Doutor Luís Guedes Martins, por aceitar orientar esta dissertação, pela disponibilidade permanente e apoio durante este processo. Agradeço o acompanhamento incansável, a ajuda para ultrapassar dificuldades e dúvidas, por me transmitir conhecimento e pela confiança depositada em mim.

À minha coorientadora, Dr^a Ana Cunha, por todo o rigor, disponibilidade e ajuda nesta jornada.

À minha família, agradeço o apoio incondicional, o carinho e por terem sido o meu grande pilar, independentemente das adversidades.

Por último, agradeço aos meus amigos, por todo o companheirismo e motivação que me deram ao longo desta jornada.

Resumo

Introdução O sistema nacional de saúde português providencia a todas as mulheres grávidas a possibilidade de realização de rastreio pré-natal. No entanto, através do rastreio realizado atualmente, verifica-se uma elevada taxa de falsos-positivos, levando à realização desnecessária de testes de diagnóstico invasivos, que acarretam diversos riscos, tais como o abortamento espontâneo. O objetivo deste estudo é avaliar as consequências económicas da implementação do teste pré-natal não invasivo (NIPT) na deteção de trissomia 21 (T21), trissomia 18 (T18) e trissomia 13 (T13), que tem um custo unitário de cerca de 260€.

Métodos Foi desenvolvido um modelo analítico para avaliar os benefícios clínicos e económicos na perspetiva do sistema nacional de saúde português e da população portuguesa atual, de forma a comparar o rastreio pré-natal atual com três estudos de caso projetados: NIPT como primeira linha, NIPT como teste contingente com rastreio combinado e NIPT como teste contingente com cálculo de risco baseado na ecografia de primeiro trimestre. Foi recolhida uma amostra de grávidas portuguesas que realizaram rastreio pré-natal para representar a população portuguesa, sendo o custo total associado ao rastreio, diagnóstico e cuidados auxiliares o principal parâmetro avaliado.

Resultados Em todos os estudos de caso apresentados observa-se uma diminuição da taxa de falsos positivos, o que implica uma diminuição dos testes invasivos realizados e, por sua vez, dos abortamentos relacionados com estes procedimentos. O NIPT como primeira linha apresenta uma melhoria na taxa de deteção comparativamente ao rastreio pré-natal atual, o que não se verifica no NIPT como teste contingente com rastreio combinado ou NIPT como teste contingente com ecografia de primeiro trimestre, que mostram uma ligeira diminuição. Contudo, enquanto o NIPT como primeira linha implica um investimento económico significativo, os restantes mostram ser opções de redução de custos.

Conclusão A implementação do NIPT como primeira linha tem demonstrado melhores resultados clínicos, no entanto, os custos associados são muito elevados. Assim, reduzir os custos do NIPT é uma medida importante para facilitar sua introdução. Quanto ao NIPT como teste contingente com rastreio combinado e NIPT como teste contingente com ecografia de primeiro trimestre, os resultados clínicos são muito semelhantes. Ambos permitem uma diminuição dos exames invasivos, mas têm uma diminuição de custos, sendo maior neste último estudo de caso.

Palavras-chave: Teste Pré-Natal Não Invasivo; DNA fetal livre das células; Triagem pré-natal

Abstract

Background The Portuguese National Health System (NHS) provided prenatal screening for all pregnant women. However, through current screening, there is a high false-positive rate (FPR), leading to unnecessary invasive diagnostic tests, which carry several risks, such as miscarriage. The aim of this study is to evaluate the economic consequences of implementing the non-invasive prenatal testing (NIPT) in the detection of trisomy 21 (T21), trisomy 18 (T18) and trisomy 13 (T13), which has a unit cost of around €260.

Methods An analytical model was developed to assess clinical and economic benefits from the perspective of the Portuguese NHS, and the current Portuguese population, in order to compare current screening with three projected case studies: NIPT as first line, NIPT as a contingent test with combined screening and NIPT as a contingent test with risk calculation based on first trimester ultrasound. A sample of Portuguese pregnant women who underwent prenatal screening was collected to represent the Portuguese population, with the total cost associated with screening, diagnosis and ancillary care being the main parameter evaluated.

Results In all case studies presented there is a decrease in FPR, which implies a decrease in invasive tests and, in turn, in procedure-related abortions. NIPT as first line shows an improvement in detection rate (DR) compared to current screening, which is not seen in NIPT as a contingent test with combined screening or NIPT as contingent test with first trimester ultrasound, which shows a slight decrease. However, while NIPT as a first line implies a significant economic investment, the rest prove to be cost-saving options.

Conclusion The implementation of NIPT as a first line has been shown to have better clinical results, however, the associated costs are very high. Thus, reducing the cost of NIPT is an important measure to facilitate its introduction. As for NIPT as a contingent test with combined screening and NIPT as contingent test with first trimester ultrasound, the clinical results are very similar. Both allow a decrease in invasive tests, but have a decrease in costs, being greater in the latter case study.

Keywords: Non-Invasive Prenatal Testing; Cell-free fetal DNA; Prenatal Screening

Lists of Abbreviations

cfDNA	Cell-Free DNA
cffDNA	Cell-Free Fetal DNA
CVS	Chorionic Villous Sampling
DR	Detection Rate
DANSR	Digital Analysis of Selected Regions
FPR	False-Positive Rate
FF	Fetal Fraction
FTS	First Trimester Screening
MPS	Massively Parallel Sequencing
MPSS	Massively Parallel Shotgun Sequencing
NHS	National Health System
NIPT	Non-Invasive Prenatal Testing
NT	Nuchal Translucency
SNPs	Single Nucleotide Polymorphisms
STS	Second Trimester Screening

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Introduction

The main goal of prenatal screening is to identify high risk pregnancies in order to diagnose abnormalities in fetuses before birth. These changes have a strong impact on families both economically and psychologically,⁽¹⁾ since congenital anomalies occur in about 2-4% of all newborns and account for 20,4% of perinatal death.⁽²⁾ More recently, attempts are being made to implement new non-invasive testing strategies to reduce the need for invasive tests, leading to a reduction in the risk of miscarriage.⁽³⁾ Furthermore, over the years, technical innovations in methodologies of analysis have seen an increase in accuracy, allowing not only the determination of fetal sex, fetal rhesus D, of many single gene disorders, but also a screening test for aneuploidies.⁽⁴⁾

The beginning of screening for birth defects began in the 1950s with the advent of obstetric ultrasound. However, it was only in the 1970s that real-time gray-scale imaging became available, which allowed the evaluation from early pregnancy. Nowadays, ultrasound has several uses, namely, determining the fetal number and gestational age, malformations assessment, and fetal wellness test, but also support in invasive diagnostic and therapeutic techniques. Moreover, in the 1950s, amniocentesis was the first prenatal chromosomal diagnostic test to be developed, which can be performed starting at 15 weeks of gestation. Later came the chorionic villous sampling (CVS) that can be performed from the 10th week.⁽⁵⁾ In the 1960s, advanced maternal age was the preferred parameter for performing an invasive test because it also increases the risk for trisomy 21, 18 and 13. Though, using maternal age alone has a very low sensitivity, around 30%, and a very high FPR, about 15%.⁽¹⁾ Although the risk of miscarriage is present, over time the procedure-related risk has become increasingly safer, around less than 1%.⁽⁶⁾ But the fact that they are associated with fetal loss has led both women and health professionals to desire the emergence of new less invasive methods with fewer associated risks.⁽⁷⁾

Afterward, two screening tests emerged through the identification of biochemical markers for fetal aneuploidies. The triple test includes alpha-fetoprotein (AFP), unconjugated estriol and beta-human chorionic gonadotropin (hCG) and has a DR of 60% and the quadruple test includes the above and inhibin A with a DR of 65%, both having a much lower FPR, around 5%.⁽¹⁾ These screening tests can be performed between the 15th and 22nd weeks of gestation.⁽⁵⁾ In the 1990s, both the nuchal translucency (NT) and the combined screening test changed prenatal screening.⁽¹⁾ NT is an echographic marker with a clear association with chromosomal anomalies, ideally being evaluated between 11 and 14 weeks with a crown-rump length of 45-84mm. A percentile above

the 95th-99th is related with adverse perinatal outcomes, with a higher risk of miscarriage, fetal death, but also congenital heart defects, other structural defects, and genetic syndromes.⁽⁸⁾ On the other hand, first trimester combined screening associates ultrasound measurements, including NT, free beta-hCG and pregnancy-associated plasma protein-A (PAPP-A)⁽¹⁾ and is carried out between 11 and 13 weeks 6 days of gestation. Thus, a risk estimate is established taking into account these parameters, but also including maternal age, weight, race, number of fetuses in the existing pregnancy and, past pregnancy history.⁽⁵⁾ However, there are other parameters, including the nasal bone, tricuspid regurgitation and, ductus venosus pulsatility index that do not indicate by themselves perinatal pathology but are associated with an increased risk of aneuploidies. In this way, they can be added to the risk estimate improving the screening result. Absence of the nasal bone, tricuspid regurgitation and, reversed a-wave in ductus venosus were observed in 59,8%, 55,7% and, 65% in trisomy 21, respectively. In contrast, in euploid fetuses these indicators were observed in 2,6%, 0,9% and, 3,2%, respectively.⁽⁹⁻¹¹⁾ For trisomy 21, 18 and 13, combined screening has an FPR of 4% and a DR of 90%, 97% and 92%, respectively.⁽¹²⁾ It allows for results at an earlier gestational age, enabling earlier reassurance, more time for interpretation of the results, and in turn, to make future decisions.^(5, 13) A better performance can be achieved by associating first and second trimester markers. Integrated screening is the combined first trimester screening coupled with the quadruple test. However, the results of the first trimester screening are not released immediately, that is, only after both studies are performed is the risk estimate calculated. Thus, the risk estimate has a more comprehensive value, allowing for an DR of about 96%, which is higher than the DRs of the other screening methods discussed.⁽⁵⁾ Although the results are much better, it has some disadvantages such as a late result, which make early reassurance of the parents impossible, and limits the time for important decisions to be made.⁽¹³⁾ Nevertheless, through a similar screening scheme, with a contingent test, this issue is overcome. First trimester markers allow stratification of cases into high, medium, and low risk, with the highest risk cases, being referred for prenatal diagnostic testing. Low risk cases have no additional screening, and finally, cases with intermediate risk, about 15% to 20%, undergo the quadruple test and the risk estimate is assessed.^(5, 13) This method allows a DR between 80% and 94%.⁽⁵⁾ Between the 18th and 22th weeks of gestation, the anatomy ultrasound can be performed, playing an important role in prenatal screening because it provides detailed anatomical information about congenital anomalies.⁽¹⁴⁾ The identification of fetal structural anomalies has a DR with a varied spectrum, between 15% and 85%, as it depends on several factors such as gestational age at the time of evaluation, the pregnant woman's BMI, the organ to be studied and the operator's experience.⁽¹⁵⁾

More recently, in 1997, it was detected DNA from the fetus in the maternal plasma during pregnancy which allowed to diagnose fetal sex through the presence of the Y chromosome.⁽¹⁶⁾ This DNA is originated in the cytotrophoblast layer of the placenta, however, although it originates from the placenta, it is mentioned to as cell-free fetal DNA (cffDNA).^(17, 18) The length of cffDNA is shorter than maternal cell-free DNA (cfDNA).⁽¹⁹⁾ Thus, it is possible to find cfDNA from both placental and maternal origin in maternal plasma, which may be useful to identify genetic anomalies in the fetus. The finding of cffDNA led to an improvement in prenatal genetic testing, which we refer to as non-invasive prenatal testing (NIPT), non-invasive prenatal screening or non-invasive prenatal diagnostic when a single-gene disorder is present. Due to factors such as confined placental mosaicism (CPM), a confirmatory invasive test is required in high-risk pregnancies because the NIPT result can be false-positive.⁽²⁰⁾ This is because cfDNA is derived from the placenta and both the placenta and the fetus have two different lineages, which diverge in the first days of embryogenesis, and thus the NIPT cannot tell them apart. CPM consists of chromosomal aberrations at the placental level that are absent in the fetus and affect 1 to 2% of pregnancies, however, its aetiology is still unknown. Development bottlenecks occur that genetically isolate individual cells, allowing clonal expansions and mosaicism. These may have a physiological genetic origin or may be due to a pathological abnormality of the normal clonal dynamics of early embryogenesis.⁽²¹⁾ However, an invasive test is not required in a single-gene disease because CPM does not interfere.⁽²⁰⁾ Thus, the NIPT is a set of molecular strategies that endorses screening, testing and possible diagnosis of chromosomal abnormalities using cffDNA.⁽²⁾

The accuracy of NIPT is dependent of a relevant parameter related to cffDNA which is the fetal fraction (FF). It consists of the portion of cffDNA in relation to total cfDNA and this fraction increases throughout pregnancy.⁽²²⁾ The cffDNA can be detected from the 6th to 7th week of gestation,⁽²⁰⁾ however, it is only after the 10th week that it is possible to identify aneuploidies.⁽²³⁾ After the delivery and the placental expulsion, cffDNA disappears from the maternal bloodstream in about 24 hours due to its rapid clearance.⁽²²⁾ The FF is a very important parameter in the NIPT, as it affects its accuracy, so a low or high FF has different meanings. The minimum level should relate to the detection limit of each NIPT analysis method.⁽²⁴⁾ However, an FF $\geq 4\%$ is advised, which has a DR of about 62%. Whereas, with an FF $\geq 9\%$, the DR reaches 100%. On the other hand, if the FF $< 4\%$, which occurs in about 1 to 3%, the test result is usually given as failed and not reported.⁽²⁵⁾ When faced with a trisomy, there is an increase on FF resulting from the supernumerary fetal chromosome. Once higher, the analysis is more accurate in distinguishing between euploid from aneuploid fetuses.⁽²⁴⁾ Though, it may be associated with complications, such as spontaneous preterm birth⁽²⁶⁾ or abnormally invasive placenta in the first trimester.⁽²⁷⁾ If

FF is low, an aneuploidy may not be detected, increasing the number of false negatives. In addition, the probability of an adverse perinatal outcome, namely pregnancy-associated hypertensive disorders, or low birthweight, is 3.5 times higher with a low FF.⁽²⁸⁾ Many factors can affect cfDNA fraction, decreasing with maternal age and high maternal body mass index, especially above 35 Kg/m². This occurs because in obesity there is an increase in circulating maternal DNA, leading to a dilution of circulating cfDNA in maternal plasma.^(28, 29) In some studies, it is thought that low FF may also be related to race, particularly in African American⁽²⁸⁾ and Asian women,⁽³⁰⁾ and with assisted reproduction techniques. On the other hand, FF increases with fetal crown-rump length⁽³¹⁾ and gestational age⁽²⁹⁾, with FF rising substantially between the 10th and 21st weeks of gestation, and from the latter week, increasing weekly by about 1%.⁽³²⁾ It also increases with placental proteins, such as free beta-hCG, PAPP-A and placental growth factor (PlGF). Taking into account these variations in the FF, NIPT is unlikely to deliver an adequate result.⁽³³⁾ In this manner, sometimes it is not possible to interpret the samples with certainty, as certain biological issues alter their interpretation, namely maternal malignancy,⁽³⁴⁾ CPM,⁽³⁵⁾ non-identical vanishing twins,⁽³⁶⁾ maternal organ transplant from a male donor,⁽³⁷⁾ or DNA copy-number variants.⁽³⁸⁾

With the assessment carried out by the NIPT, an increase in the chromosomal content in maternal cfDNA can be identified, due to a possible trisomic cell line in the fetus.⁽²⁰⁾ For trisomy 21, cfDNA analysis in maternal plasma has a DR higher than 99% with a false-positive rate (FPR) under 0,1%, while for trisomy 18 and 13 the DR is about 96% and 91%, respectively, with a combined FPR of 0,26%.⁽³⁹⁾ So, NIPT has been shown to have a lower FPR, a higher sensitivity, and a higher positive predictive value compared to standard screening.⁽⁴⁰⁾ Regarding trisomy 21, there is evidence indicating that cfDNA screening is a highly accurate test compared to other methods integrating maternal age, first- or second-trimester ultrasound findings and serum biochemical analysis. Since testing can be performed early in pregnancy, it can provide reassurance for most parents that their fetus has a low possibility of them being trisomic.⁽³⁹⁾ Thus, invasive test incurs the risk of miscarriage, while NIPT does not pose the same risk.⁽²⁰⁾ On the other hand, parents with fetuses who are carriers of such mutations have the option to terminate the pregnancy safely.⁽³⁹⁾ Although the implementation of NIPT has led to a reduction in invasive tests, this implementation has brought some negative consequences.⁽⁴¹⁾ Physicians have less opportunity to perform invasive procedures, which leads to higher miscarriage rates.⁽⁴²⁾ Furthermore, there is also a decrease in the detection of severe deletions syndromes that cause severe disabilities.⁽⁴³⁾ Nevertheless, regardless of the advances that may follow, invasive test will continue to play a fundamental role in prenatal diagnose.⁽⁴²⁾

Analysis methods for the NIPT have been based on three main techniques, namely, the analysis of the entire genome, of specific regions and of single nucleotide polymorphisms (SNPs). These techniques allow the identification of copy-number variants (CNVs), microdeletions and chromosomal abnormalities, including trisomy 21, 18 and 13 and sex chromosome defects. In general, in these NIPT approaches, the number of fragments of a given chromosome is compared to a disomic control. The ratio by this comparison in a euploid fetus will be approximately 1. However, in the case of trisomy, there is an increased fetal fraction due to the increased release of supernumerary chromosome fragments in maternal plasma.⁽²⁾

One of the techniques used is massively parallel sequencing (MPS) that sequences the whole genome and allows the detection of fetal aneuploidies. It presents important parameters such as sequencing depth and coverage. The sequencing depth is the number of reads of a given base sequenced in the reference genome. It is found that there are different depths considering the diagnostic purposes, however, for the diagnoses of fetal aneuploidies, only a low depth is needed. As for the sequencing coverage, this corresponds to the percentage of target bases that are sequenced a certain number of times.⁽⁴⁴⁾ In this technique, cfDNA sequencing is performed in the maternal plasma and, subsequently, each molecule is attached to its corresponding chromosome and quantified. In this way, a proportional representation is calculated for each chromosome, and after the comparison with healthy pregnant women with euploid fetuses, the presence of aneuploidies can be assessed. In addition to detecting trisomies 21, 18 and 13 and sex chromosome aneuploidies, MPS can also be used to detect subchromosomal duplications and deletions if sufficient DNA sequencing depth is available. On the one hand, this technique allows the detection of both chromosomal and subchromosomal anomalies. However, its cost is high and, when sequencing the whole genome, some regions are identified but have no clinical interest.⁽⁴⁵⁾

Instead, there is an MPS-relative technique called massively parallel shotgun sequencing (MPSS) that consists of the amplification and sequencing of cfDNA in maternal plasma, not requiring the separation of fetal and maternal DNA. In this technique, the number of a particular chromosome of interest is determined and then compared to its corresponding reference value. The MPSS has a high positive predictive value, essentially for trisomy 21 with less success for trisomy 18 and 13. As a disadvantage, in addition to its high cost, it also requires a fetal fraction of at least 4%,⁽⁴⁶⁾ as this is directly related to chromosomal dosage. On the other hand, since chromosome 21 only represents 1,5% of the entire genome, millions of reads are needed to ensure a sufficient count of chromosome 21.⁽⁴⁷⁾ More specifically, in a pregnant woman with a fetus diagnosed with trisomy

21, using this technique, about 10 million reads are performed, whereas only 32000 sequences of chromosome 21 have been used for aneuploidy detection.⁽⁴⁸⁾

There is another method available with a specificity of about 96% that allows a highly multiplexed sequencing of selected loci of chromosomes of interest that it is the digital analysis of selected regions (DANSR). It is a technique with advantages over MPSS because it has a lower cost, less complexity and better throughput. When using MPSS, detecting an aneuploidy requires about 25 million reads, allowing 4 and 6 samples to be evaluated simultaneously. However, when using DANSR, only 1 million reads are required, allowing 96 samples to be evaluated. In addition, DANSR allows genotyping of individual polymorphic loci that cannot be obtained using MPSS.^(46, 47)

Lastly, SNPs consist of a unique variation in a particular nucleotide and are the most common human DNA genetic variation. This technique is based on the amplification and analysis of SNPs between alleles at a locus through a highly multiplexed-PCR, determining chromosome copy number and searching for a specific pattern in allelic measurements. It is possible to detect triploidy, dizygotic twin ploidy, maternal mosaicism, and uniparental disomy, and since it is performed by multiplex sequencing, it is possible to analyze more cases per run. However, it cannot be applied in IVF pregnancies with egg donation, in cases of bone marrow transplantation, or when there is consanguinity, due to lack of parental heterozygosity.^(45, 46)

In this article, Harmony™ Non-Invasive Prenatal Test is used, which is a screening test that uses DANSR. It identifies specific sequences on chromosomes 21, 18 and 13 and quantifies them. In addition, identifies SNPs on chromosomes 1 to 12 in order to assess fetal fraction.⁽⁴⁹⁾

Currently, in Portugal, pregnant women undergoing prenatal screening are offered combined screening in first trimester and when arriving late the quadruple test (in the second trimester). So, the major purpose of this article is to assess the clinical relevance and the cost consequences of using the Harmony™ Non-Invasive Prenatal Test in public healthcare systems, considering short-term costs and outcomes, with long-term outcomes included in a secondary analysis. Thus, we compared the results from applying NIPT as first line for all pregnant women presenting for screening, NIPT as contingent test with combined screening and NIPT as a contingent test with risk calculation based on first trimester ultrasound.

Methods

An analytical model in excel was developed in order to assess the consequences and simulate the financial outcome of the introduction of the NIPT in the prenatal screening currently offered by the Portuguese national health system to pregnant women. This model was developed from the perspective of the Portuguese NHS, accounting the ways and management of pregnant women and their associated costs according to the typology developed in Portugal. In this model, pregnant women were followed from the beginning of pregnancy until delivery through prenatal screening, invasive diagnostic procedures and ancillary care that might be necessary. Thus, a sample of Portuguese pregnant women who underwent prenatal screening was collected, considering their age and the type of screening performed (first or second trimester screening), in order to assess the epidemiology of the target population, representing thus the current situation of the country.

The economic comparison is made between the currently available prenatal screening and three study cases where NIPT is implemented in different ways (NIPT as first line screening, NIPT as contingent test with combined screening, and NIPT as contingent test with risk calculation based on first trimester ultrasound). The main outcome of this study is the total cost of implementing NIPT, through costs associated with screening (NT ultrasound, first and second trimester serum screening, and NIPT), costs of invasive diagnostic tests (CVS and amniocentesis, both with sensitivity and specificity of 100%) and ancillary care costs (procedure-related miscarriage, procedure-related amniotic fluid leak, rare procedure-related complications, termination of pregnancy, additional medical consultation, and genetic counselling) (Table I). It is considered that approximately 0.5% of women who start screening will have a NT > P99 or a major structural anomaly, thus presenting an indication for an invasive study. Knowing that not all women at high risk of having a fetus with trisomy undergo an invasive study, it is considered that the percentage of obtaining invasive tests after a positive first or second trimester result or a positive NIPT is 87.50%.⁽⁵⁰⁾ Regarding auxiliary care, it is considered that the risk of miscarriage related to the procedure is 0.5%,⁽⁵¹⁾ while procedure-related amniotic fluid leak and rare procedure-related complications (such as, prolonged rupture, infection, fetal-maternal hemorrhage, Rh sensitization (no antiD), and fetal trauma) have a risk of 1%. On the other hand, it is considered that in cases of fetuses detected with trisomy, there is a 95.45% probability that the pregnant woman will request the termination of the pregnancy.⁽⁵⁰⁾ A complementary medical consultation is offered to all pregnant women who are at increased risk for trisomy, and it is estimated that 15% of these will require genetic counselling. As for the clinical outcomes, the number of pregnant women tested, the DR for each trisomy according to the sensitivities and specificities shown in table II, as well as

the number of invasive tests for diagnosed aneuploidy and the number of invasive tests for false positives were evaluated.

Thus, using this model and considering the characteristics of the Portuguese population, it was possible to estimate the difference in costs associated with the different proposals for implementing the NIPT in the current prenatal screening program.

Population

Over the years, the Portuguese population has undergone changes which have also been seen with the number of births in the last 20 years. According to the *Instituto Nacional de Estatística*, in 2000 there were 119368 births,⁽⁵²⁾ with a significant decrease, with 83784 births in 2020.⁽⁵³⁾ We were faced with the absence of statistical data regarding the number of pregnancies annually in Portugal, therefore, so we analyzed a population of 1151 pregnant women who were referred to the largest Portuguese maternity hospital (Table III). Thus, through the number of births, voluntary interruptions of pregnancy and the percentage of spontaneous fetal loss, we estimated the number of pregnancies in the year 2020.

Of the referenced pregnant women, there was an increase in the percentage of pregnant women aged over 35, as well as the average age. Only 883 underwent prenatal screening, which represents 76.72% of pregnant women, due to several factors, namely, the lack of desire for prenatal screening, its performance in private institutions or an uncompleted screening. However, from the screenings carried out, there is a predominance of screening in the first trimester, as expected, around 90%. Pregnant women who undergo screening in the second trimester, around 10%, are usually due to a late referral or a more advanced gestational age than initially expected, making it no longer possible to perform prenatal screening in the first trimester.

Comparison of case studies

In this study, we compare three case studies: (a) NIPT as first-line screening; (b) NIPT as contingent test with combined screening; and (c) NIPT as contingent screening with risk calculation based on first trimester ultrasound (Figs. 1, 2 and 3).

For the first study case (a), NIPT as first-line screening would replace biochemical markers of first trimester (PAPP-A and beta-hCG). However, first trimester ultrasound remains, as it provides important parameters for the evaluation of other possible chromosomal anomalies. Thus, we would implement NIPT in all pregnant women who did not have nuchal translucency above P99 or other a structural anomaly when performing the first trimester ultrasound. If the NIPT result is positive or indeterminate, an invasive test (CVS or amniocentesis) is recommended to confirm the diagnosis. In case of a negative result, no further studies are carried out.

Second, NIPT as contingent test with combined screening (b) consists of initially performing combined screening (first trimester ultrasound and serological markers) in all pregnant women presenting for screening. Maternal age and modifying factor, such as ethnicity, weight, smoking status, the gestational age at which the sample is collected, and others, are considered for the risk calculation. A cut-off of $>1:100$ was set for high-risk pregnancies taking into account the prevalence of trisomy 21 in general population. If pregnant women are at high risk, they are proposed for an invasive study. If they present an intermediate risk (between $1:100$ and $1:800$), the NIPT would be offered, following the process described in the previous case study (a).

Lastly, for the NIPT as a contingent test with risk calculation based on first trimester ultrasound (c), the risk calculation is based only on ultrasound markers, such as nuchal translucency and nasal bone. Thus, the cut-off point considered for high-risk pregnancies is different ($>1:1,000$), since the sensitivity of screening using only first-trimester ultrasound is slightly lower compared to the second case study. Therefore, if there are high-risk pregnancies, there is an indication for NIPT, otherwise no further study is required.

Results

Regarding the prenatal screening currently carried out, 70,084 pregnant women start screening in the first trimester with ultrasound, which represents 90%. However, 351 of these pregnant women soon had an indication for an invasive study due to a major structural anomaly or NT > P99. Thus, only 69,733 complete the first trimester screening by doing the serum biochemistry screen. Of the remaining pregnant women, representing 10%, 7,787 carry out screening in the second trimester. Of these screenings, 5,535 invasive studies are performed.

With the introduction of NIPT as a first line, screening begins with the performance of 70,084 first trimester ultrasounds, and first and second trimester serum screening is not performed. The 10% of pregnant women who undergo second trimester screening start their screening right away with the NIPT test. Thus, 77,521, undergo NIPT. (Fig. 4). In this way, there is a decrease in the performance of invasive diagnostic tests by approximately 93%, decreasing from 5,535 to 394 tests performed. Furthermore, knowing that many invasive diagnostic tests were performed due to false positives, with this study case, there is a 98% decrease in tests performed unnecessarily for this reason. Thus, by reducing its performance, it allows a significant reduction of abortions resulting from these procedures from 28 to 2, but also a reduction of other procedure-related complications from 111 to 10. On the other hand, there was a significant global increase in the DR of the trisomies, going from 83% to 99%, most pronounced in trisomy 21 with an increase in the DR of 19%. However, there was also an increase of 7% for trisomy 18 and 15% for trisomy 13. Finally, using NIPT tests decreases the number of invasive tests per diagnosed aneuploidy by 94%.

Regarding the economic outcomes of the first case study, NIPT as the first line, it appears that the total cost of screening and diagnosis increases by €9,935,688 compared to standard screening, from €13,832,569 to €23,768,257 (Fig. 5). While there is a decrease in costs associated with first trimester biochemical screening, second trimester screening, and invasive testing, the cost associated with performing NIPT leads to this significant increase in costs. As for the costs associated with ancillary care, which include procedures related to miscarriage, amniotic fluid leakage, rare complications, pregnancy interruptions, genetic counselling and additional medical visits, there is a slight decrease in expenses of about half a million euros. Regarding total costs, considering screening, diagnosis and ancillary care, there is an increase of €9,440,066. However, although costs have increased, this is due to the prenatal screening program, as there is a decrease in costs associated with invasive tests and ancillary care. Thus, it appears that the cost of screening per pregnant woman is €309, increasing €121 compared to normal screening.

Regarding the clinical results of this second case study, considering that the current prenatal screening and this case study start in the same way, it appears that the number of prenatal screenings performed is the same, that is, 70,084 undergo a first trimester ultrasound, 69,733 perform a serological biochemical study, and 7,787 perform a second trimester screening. However, in this model 5,926 NIPT tests are performed (Fig. 6). There is also a decrease in the number of invasive tests performed, although to a lesser extent in relation to the use of NIPT as the first line, around 89%, decreasing from 5,535 to 620. Thus, there is also a decrease in invasive tests performed unnecessarily due to false positives by approximately 93,5%, allowing, again, a decrease of about ten times in abortions related to these procedures and in other procedure-related complications. On the other hand, using NIPT as a contingent test, leads to a reduction of about 1% on the overall trisomy detection. It appears that the detection rate of trisomy 21 does not change, remaining at 81%. However, in both trisomy 18 and trisomy 13, a decrease in the detection rate of 2% and 4%, respectively, is observed. Finally, with this case study it is observed a decrease in the number of invasive tests for diagnosed aneuploidy by 89%.

In the second case study presented, NIPT as contingent test with combined screening, the total cost of screening and diagnosis decreased by €3,101,212 compared to standard screening, from €13,832,569 to €10,731,357 (Fig. 7). It was found that this cost reduction is essentially associated with the decrease in the number of invasive tests performed, as the costs associated with ultrasounds, first trimester biochemical screening and, second trimester screening are the same as for standard screening. In addition, the cost of ancillary care was reduced by €227,826, primarily due to procedure-related amniotic fluid leakage, and rare procedure-related complications. Instead, there is an increase in costs associated with genetic counselling and additional medical consultation for high-risk screens. As for total costs, although there was an increase in costs related to screening, there was a decrease associated with invasive tests and ancillary care, with a decrease of €3,329,038. This way, the cost associated with prenatal screening per pregnant woman is reduced to €145.

Regarding the third case study, 70,084 pregnant women, representing 90% of the women who receive prenatal care, are initially submitted to first trimester ultrasound, with no serological biochemical screening being performed. Of the remaining pregnant women, 7,787 undergo screening in the second trimester. Taking into account the similarity that exists between the screening sensitivity of the second and third case studies, it is assumed that by not performing the biochemical screening and by increasing the cut-off to 1:1000, there is an increase of about 25% in the NIPT tests performed, rising to 7,408 (Fig. 8). Due to this increase, it is estimated that some

more invasive tests will be performed. However, compared to standard screening, there is a substantial decrease in invasive tests, which is also around 86%, from 5,535 to 775. It appears that the use of NIPT reduced the number of unnecessary invasive tests due to false positives by approximately 91.5%, similar to the second case study. In addition, there is also evidence of an overlap in the DR of the overall trisomies and a decrease in the number of invasive tests for diagnosed aneuploidy.

Regarding the economic outcomes related to this case study, there is a considerable decrease in the total cost of screening and diagnosis by € 7,618,171 compared to standard screening, from € 13,832,569 to € 6,214,398 (Fig. 9). Although there are expenses with performing NIPT, this reduction in costs is due to the absence of biochemical screening in the first trimester and the decrease in invasive tests. As for the costs associated with ancillary care, it is estimated that these are similar to those spent in the case study of NIPT as a contingent test with combined screening, taking into account the similarity of sensitivities. In this way, there is a decrease of €7,845,997 when considering the total costs compared to standard screening, and this decrease is associated with the lower number of screening procedures performed. Thus, the cost associated with screening per pregnant woman is around €87, decreasing €101.

Discussion

There is a constant search for new screening methods with increasingly higher detection rates, in order to identify the greatest possible number of trisomies, reducing invasive tests and their consequences. The NIPT is a screening test that is currently not available to be carried out through the Portuguese public NHS, being only accessible in the private health sector for pregnant women who intend to carry it out. Thus, the main purpose of the study is to assess the financial outcome of including the NIPT in the screening model currently practiced in the Portuguese NHS. This study may help in a possible paradigm shift in prenatal screening and diagnosis carried out in Portugal, allowing, in turn, a better perception of what measures could be adopted, bearing in mind the relevance and sustainability of the introduction of NIPT. For this, three case studies are presented that are compared with the standard screening, these being NIPT as first line, NIPT as contingent test with combined screening and NIPT as contingent test with risk calculation based on first trimester ultrasound.

Given the results presented above, it appears that NIPT as a first line presents a significant increase in the number of trisomies detected and a decrease in invasive tests performed, leading to a decrease in abortions related to these procedures. However, it presents an increase in the financial burden on the NHS by about 64% of total costs compared to current screening. As for the case study that we present with NIPT as a contingent test with combined screening, there is a slight decrease in detected cases, although for T21 there is no change. There was also a reduction in the number of invasive tests performed, therefore, abortions related to the procedure decreased, and there was also a 23% decrease in costs. Considering NIPT as contingent test with first trimester ultrasound, the number of cases detected will be identical to that of NIPT as contingent test with combined screening, there is a similar reduction in the number of invasive tests and abortions resulting from them. However, the savings seen in this case study are higher, with a 54% decrease in total costs.

In our analysis, it appears that the use of NIPT as a first line presents better clinical results compared to the other two models, since in addition to having a better DR, it presents a greater decrease in the invasive tests performed and, therefore, less consequences for the pregnant woman and the fetus. However, the implementation of NIPT as contingent test with combined screening or NIPT as contingent test with first trimester ultrasound also shows good clinical results. Although there are a small number of cases that may go undetected, the reduction in invasive exams is also very significant. Thus, evaluating the clinical results of the three models, the use of NIPT as the first line is the most advantageous. However, as this is a financial impact study,

it is necessary to consider the costs associated with this improvement in clinical outcomes. Therefore, the costs associated with NIPT as first line screening and diagnosis are much higher than current costs. Even with ancillary costs decreasing, the total value remains substantially higher. On the other hand, regarding NIPT as contingent test with combined screening or with ultrasound in the first trimester, the costs of screening and diagnosis are lower, with emphasis on the latter case. As far as ancillary costs are concerned, these are slightly higher compared to NIPT as a first line. However, evaluating the total costs, the most financially appealing case study turns out to be NIPT as contingent test with risk calculation based on first trimester ultrasound.

In view of the new health measures available, the parameters considered most important are cost-effectiveness, effectiveness, and budgetary impact. Thus, study methodologies such as cost-effectiveness studies were created to carry out this evaluation. This type of study assesses a long period of time, combining clinical and economic results.⁽⁵⁴⁾ However, considering that cost-effectiveness studies do not have the necessary characteristics for those who make health decisions in the face of available budgets, financial impact studies have emerged to complement and fill these gaps.^(55, 56) This type of study makes it possible to estimate the financial impact, in the short and long term, of the implementation of a new measure, considering the current population, thus assessing its sustainability.⁽⁵⁴⁾

Several financial impact studies were previously carried out in other countries, however, there are several differences between them, from the cost of the NIPT to parameters of the designed models, making their comparison more difficult. In Italy,⁽⁵⁷⁾ the implementation of NIPT as a contingent test with combined screening at the same cost as our study was evaluated, with a risk cut-off $\geq 1:1000$, proving to be a cost-saving option. The same evaluation was carried out in Canada,⁽⁵⁸⁾ however, the cost of NIPT is approximately twice as high, and it is applied with a risk cut-off $\geq 1:300$. In this study, it was shown that the application of NIPT as a contingent test with combined screening was a cost-saving or cost-neutral option. A cost-effectiveness study from Spain⁽⁵⁹⁾ considered NIPT as a first line test, but also as a contingent test. The cost of NIPT is also higher and the cut-off for the base case was $\geq 1:270$, with NIPT as a second line being the most affordable option. When carrying out this same evaluation with the much more affordable NIPT price, it was found that using NIPT as the first line was still more expensive, however, the difference was much smaller. On the other hand, as a second line there were greater savings of resources. When evaluated with a cut-off $\geq 1:500$, the effectiveness was better, but the costs also went up. No study was found that evaluated the financial impact of NIPT as contingent test with risk calculation based on first trimester ultrasound.

This study has some limitations, as although it is useful to simulate the costs and possible benefits and trade-offs of introducing the NIPT, so that case studies can be created with parameters appropriate to the population, it is necessary to make some assumptions. Within these limitations we first have which the population sample presented is not real, but there is a representation of the Portuguese population from a sample taken from the largest Portuguese maternity hospital, due to the lack of such statistical data in Portugal. Finally, the costs related to the investment required to implement the NIPT in the Portuguese NHS are not considered, with only the costs associated with screening being considered. Therefore, costs would be higher in all case studies, however, this difference in costs would be the same for all, not differing in the analysis between them. On the other hand, compared with the current screening, the introduction of NIPT may not seem so economically advantageous, however, the costs related to the implementation of NIPT dissolve over the following years. Considering the limitations of the study, they are not predictable to have an impactful difference on the results presented. On the other hand, it is considered that the strong point of the study is the assessment of the costs of both screening, diagnosis and ancillary care, but also the ability to assess costs not only related to T21, but also including T18 and T13.

Conclusion

The implementation of NIPT in the Portuguese NHS, regardless of the model to be adopted, has benefits. There is always a decrease in FPR, so there is a smaller and better selection of pregnant women who must undergo an invasive study, entailing less risks, such as abortions related to these procedures, but also other complications that, although rare, may eventually occur. Comparing with current screening, although NIPT as first line has better clinical results, with better detection rates, the financial impact on the health system is very high. However, making the cost of the NIPT more affordable would possibly make this model a double benefit. On the other hand, both the NIPT as a contingent test with combined screening and the NIPT as contingent test with risk calculation based on first trimester ultrasound, although they have a small decrease in the detection rate, they present the other clinical benefits, with better management of pregnant women, therefore the associated costs are much lower.

Attachments

Table I Costs inputs

Description	Value
Screening Costs	
First trimester serum screening	€72,40
NT ultrasound	€46,24
Second trimester screening	€40,54
Harmony™ NIPT test	€260,00
Diagnosis Costs	
CVS	€944,45
Amniocentesis	€944,45
Ancillary Care Costs	
Treatment for miscarriage	€105,18
Termination of pregnancy	€914,39
Genetic Counselling	€181,69
Additional doctor's visit	€24,48
Procedure-related leakage	€3 514,54
Rare complications	€1 264,54

Table II Testing parameters

		T21	T18	T13
FTS ⁽⁶⁰⁾	<i>Sensitivity (95% CI)</i>	88.00 (50.00–100.00)	90.00 (33.00–100.00)	78.00 (57.00–100.00)
	<i>Specificity (95% CI)</i>	95.00 (91.00–99.00)	94.00 (94.00–100.00)	92.00 (92.00–96.00)
STS ⁽⁶⁰⁾	<i>Sensitivity (95% CI)</i>	80.50 (60.00–100.00)	63.50 (53.00–86.00)	50.00 (1.00–99.00)
	<i>Specificity (95% CI)</i>	90.50 (46.00–96.00)	99.25 (99.64–100.00)	64.00 (61.00–67.00)
Harmony NIPT Test ⁽⁶¹⁾	<i>Sensitivity (95% CI)</i>	99.30 (97.90–99.80)	97.40 (93.40–99.00)	93.80 (79.90–98.30)
	<i>Specificity (95% CI)</i>	99.96 (99.92–99.98)	99.98 (99.95–99.99)	99.98 (99.94–99.99)

Table III Characterization of the population

Parameter	Value	Source
Pregnant women referred		1151
Average age		31,30
Pregnant women aged 35 years or older		29,00%
Pregnant population attending pregnancy follow-up		76,72%
1st trimester screening tests performed		90,00%
2nd trimester screening tests performed		10,00%
Both 1 st and 2 nd trimester screening performed		0,00%
Spontaneous fetal loss		7,47%
Number of births in 2020	83784	(53)
Voluntary induced abortion	14369	(62)
Total pregnancies	104413	Estimated
Twin pregnancies	2,79-4,5%	(63) (64) (65) (66)
Pregnant women who have prior history of trisomy 21	1,0%	(67)

Table IV Cost results of NIPT implementation

	Standard of Care	Intervention (Harmony™ NIPT Test)		
		First line NIPT screening	Contingent NIPT screening	NIPT with first trimester ultrasound
Ultrasound	€3,240,684	€3,240,684	€3,240,684	€3,240,684
FTS testing	€5,048,669	-	€5,048,669	-
STS testing	€315,685	-	€315,685	€315,685
NIPT testing	-	€20,155,460	€1,540,760	€1,926,080
Total screening costs	€8,605,038	€23,396,144	€10,145,798	€5,482,449
Invasive testing	€5,227,531	€372,113	€585,559	€731,949
Total screening and diagnosis costs	€13,832,569	€23,768,257	€10,731,357	€6,214,398
Procedure related miscarriage	€2,911	€207	€326	€326
Procedure related leakage of amniotic fluid	€194,530	€13,847	€21,790	€21,790
Procedure related rare complications	€69,992	€4,982	€7,840	€7,840
Pregnancy terminations	€231,288	€288,019	€229,543	€229,543
Genetic counseling	€172,398	€12,272	€178,401	€178,401
Additional doctors visit	€154,853	€11,023	€160,246	€160,246
Total ancillary care costs	€825,972	€330,350	€598,146	€598,146
Total costs	€14,658,541	€24,098,607	€11,329,503	€6,812,544
Cost per screened woman	€188	€309	€145	€87

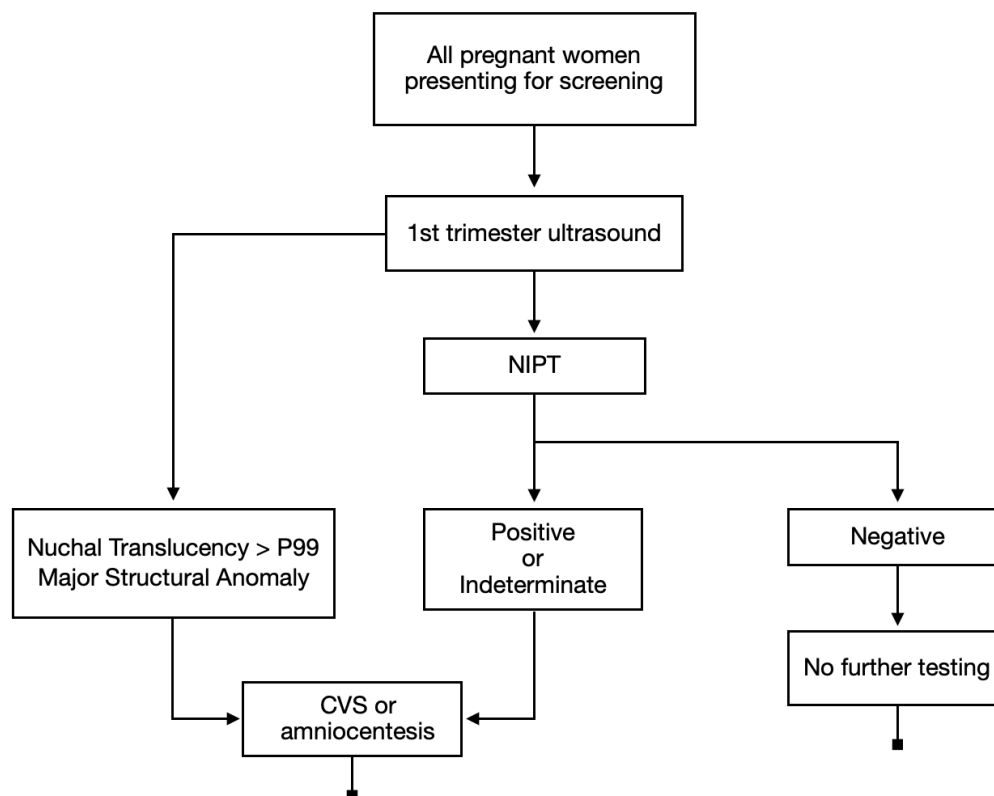


Fig. 1 Diagram illustrating the application of NIPT as first-line screening

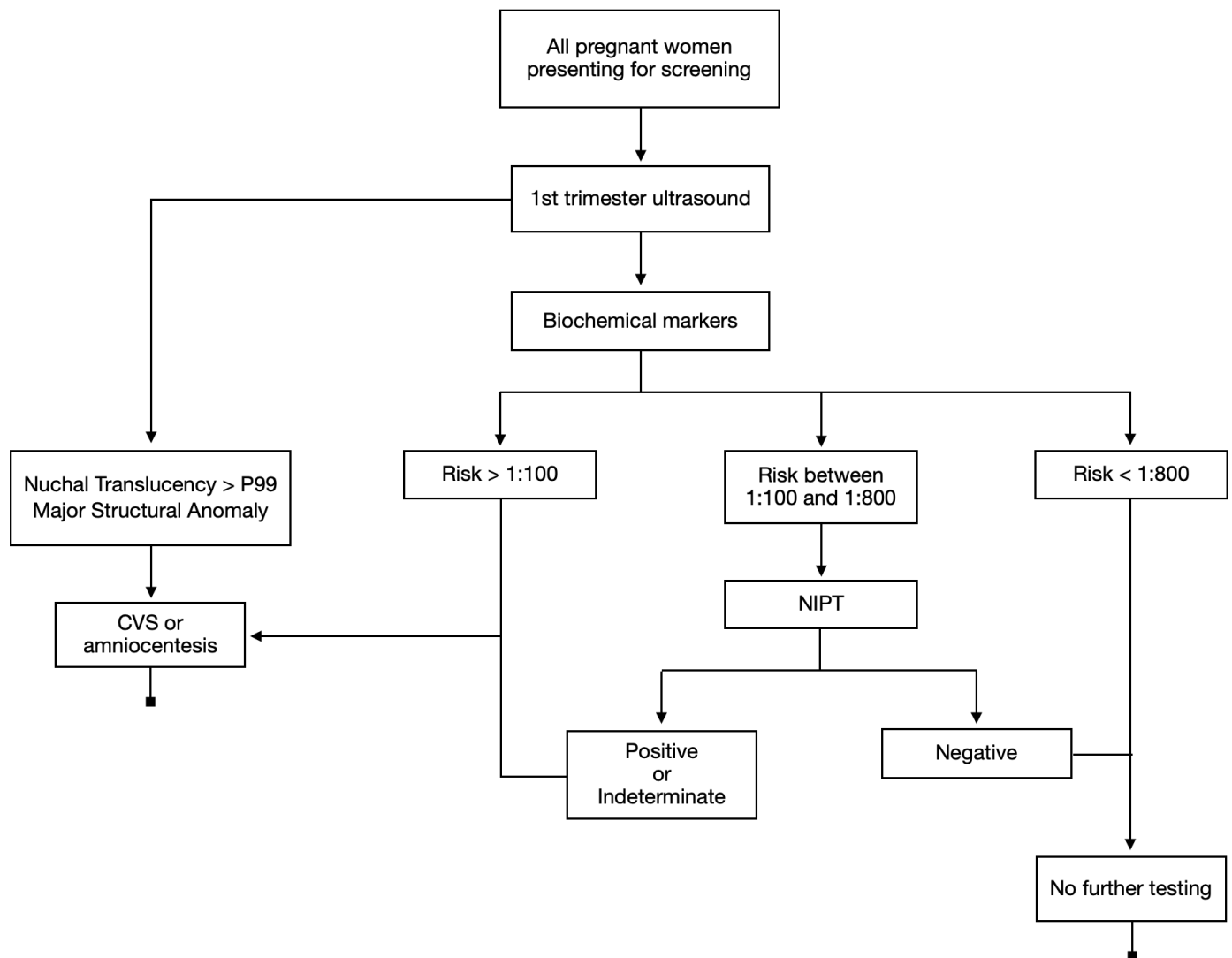


Fig. 2 Diagram illustrating the application of NIPT as contingent screening with biochemical markers

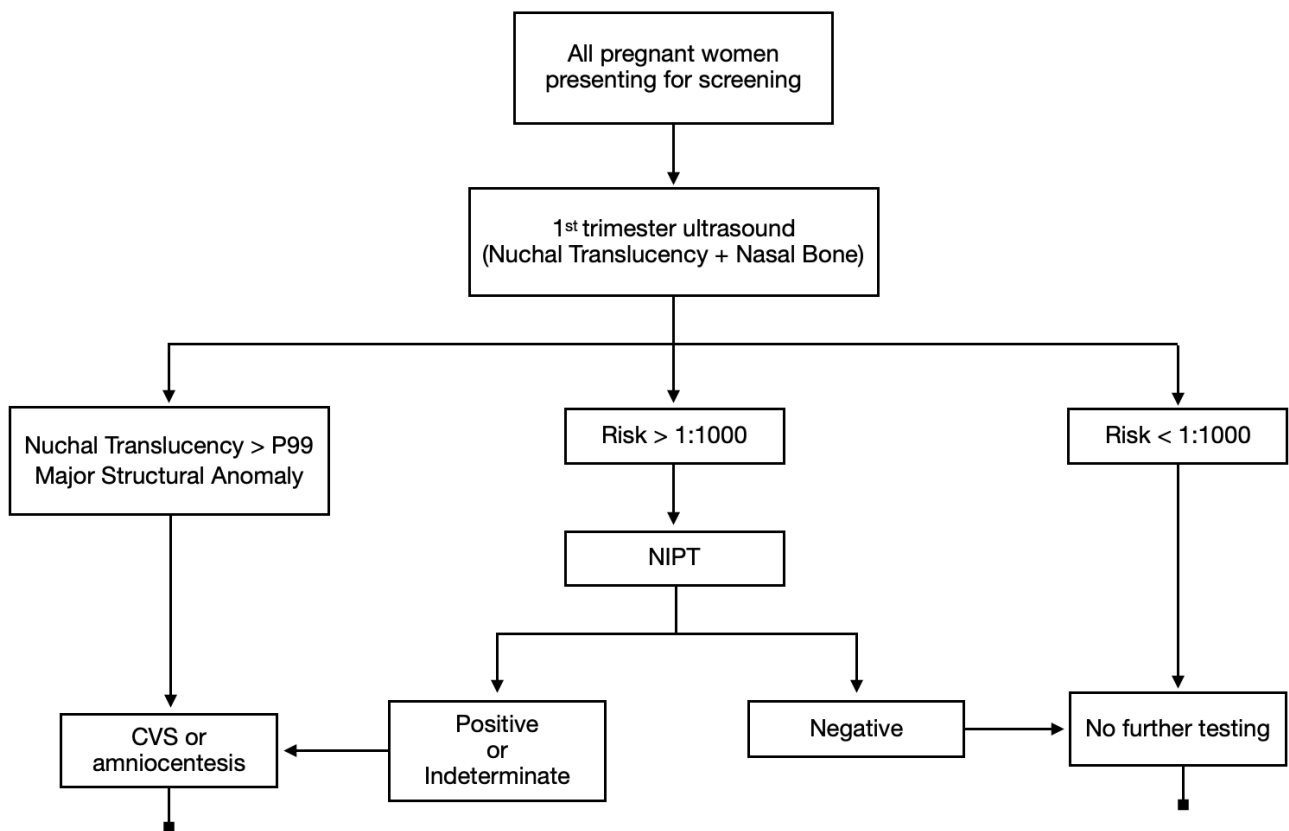


Fig. 3 Diagram illustrating the application of NIPT as contingent screening with first trimester ultrasound

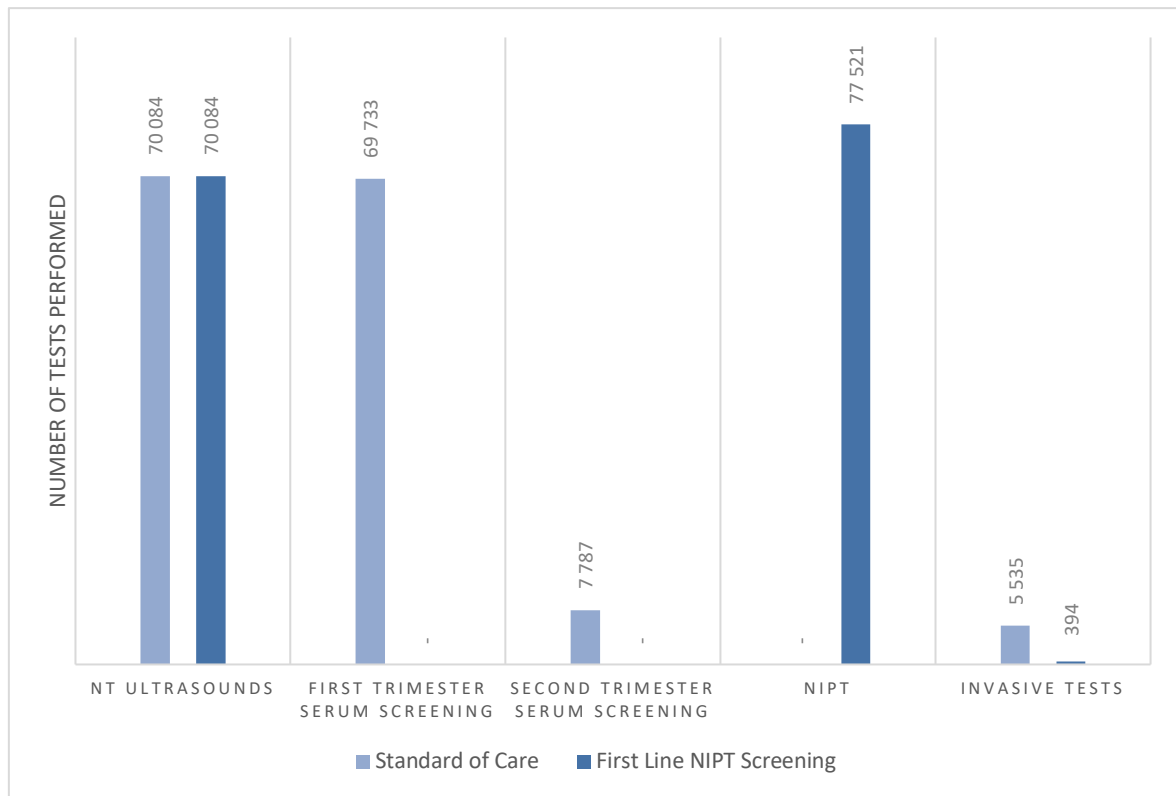


Fig. 4 Number of pregnant women tested in NIPT as first line (In standard of care, 70,084 first trimester ultrasounds are performed, with 69,733 collecting the biochemical studies to complete the first trimester screening. 7,787 pregnant women are tested with second trimester serum screening. No NIPT tests are performed, and there are 5,535 invasive tests that are completed. In first line NIPT screening, 70,084 first trimester ultrasounds are performed and no first or second trimester serum screening is performed. 77,521 pregnant women undergo NIPT testing, with 394 invasive studies being performed.)

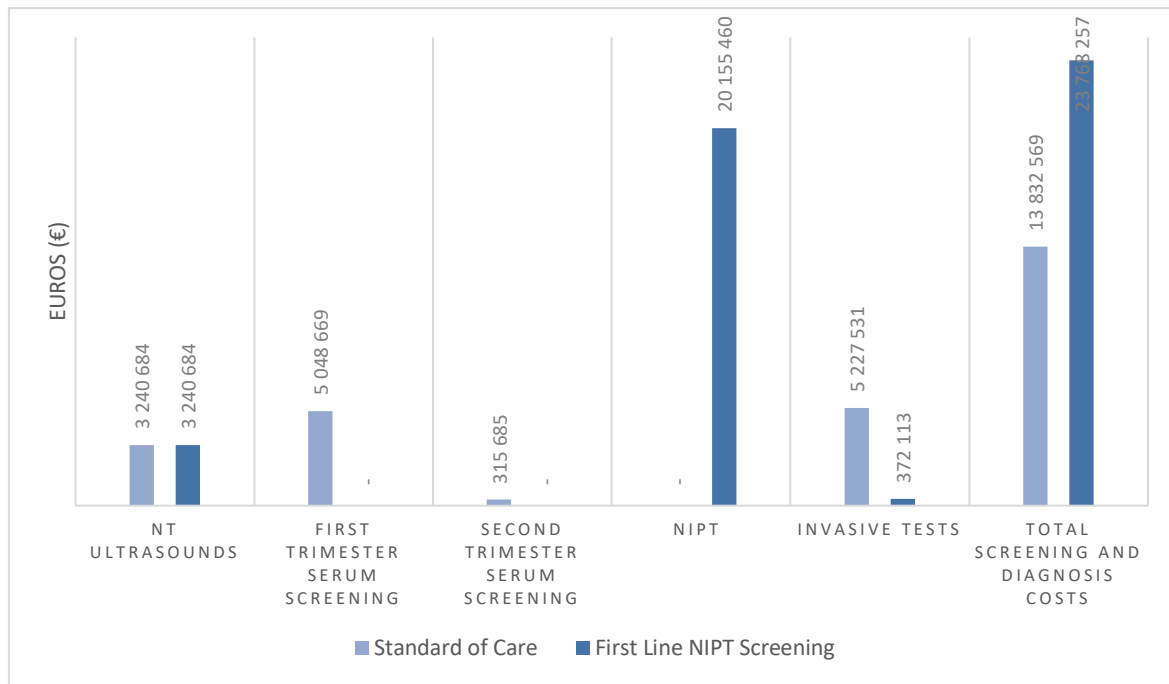


Fig. 5 Testing costs in NIPT as first line screening (In standard of care, €3,240,684 is spent on carrying out first trimester ultrasounds, €5,048,669 on the biochemical study to complete the first trimester screening and €315,685 on the second trimester serum screening. There are no expenses associated with the NIPT but carrying out invasive tests costs €5,227,531. The total screening and diagnosis costs is €13,832,569. Regarding the costs of first line NIPT screening, €3,240,684 is spent on carrying out first trimester ultrasounds, but there are no costs associated with first or second trimester serum screening. €20,155,460 is spent on NIPT and €372,113 on invasive tests. The total amount for screening and diagnosis is €23,768,257.)

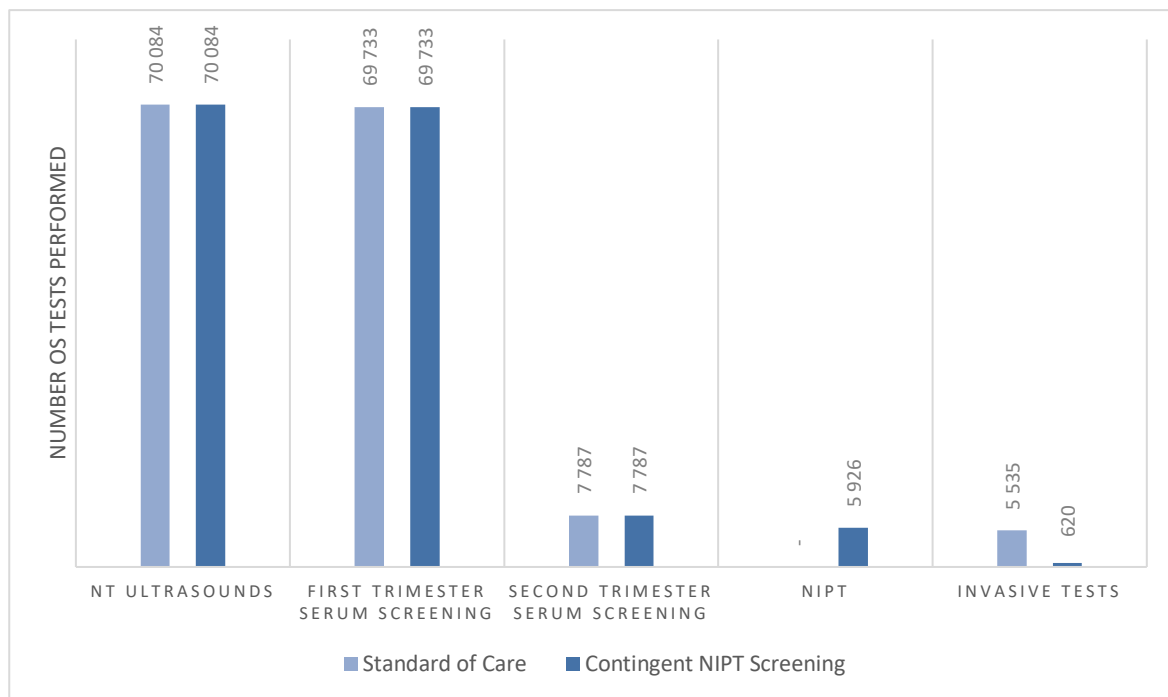


Fig. 6 Number of pregnant women tested in NIPT as contingent screening with biochemical markers (In standard of care, 70,084 first trimester ultrasounds are performed, with 69,733 collecting the biochemical studies to complete the first trimester screening. 7,787 pregnant women are tested with second trimester serum screening. No NIPT tests are performed, and there are 5,535 invasive tests that are completed. In contingent NIPT screening, 70,084 first trimester ultrasounds, 69,733 first trimester serum screenings and 7,787 second trimester serum screenings are also performed. 5,926 NIPT tests and 620 invasive studies are carried out.)

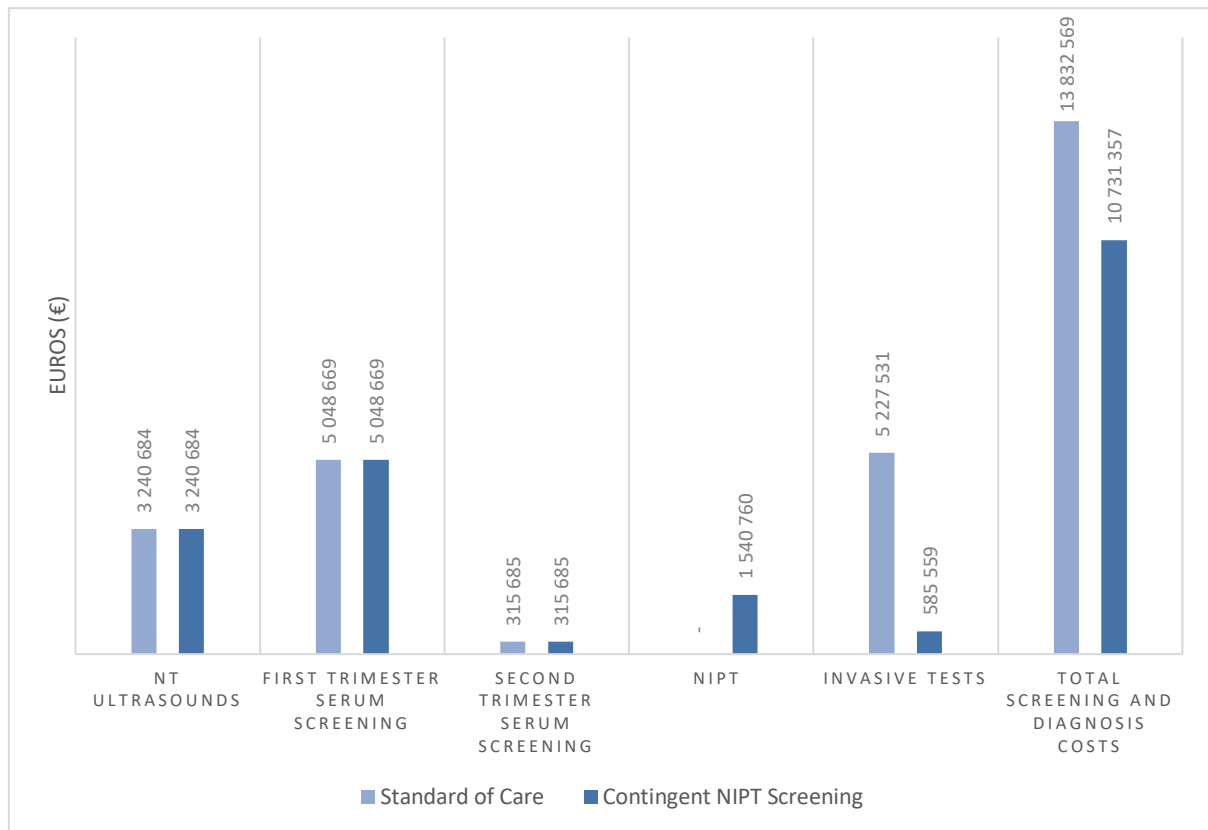


Fig. 7 Testing costs in NIPT as contingent screening with biochemical markers (In standard of care, €3,240,684 is spent on carrying out first trimester ultrasounds, €5,048,669 on the biochemical study to complete the first trimester screening and €315,685 on the second trimester screening. There are no expenses associated with the NIPT but carrying out invasive tests costs €5,227,531. The total screening and diagnosis costs is €13,832,569. Regarding contingent NIPT screening, costs of ultrasound, first and second trimester serum screening are equal to the standard of care. There is a cost of €1,540,760 associated with NIPT tests and €585,559 for invasive tests. The total amount for screening and diagnosis is €10,731,357.)

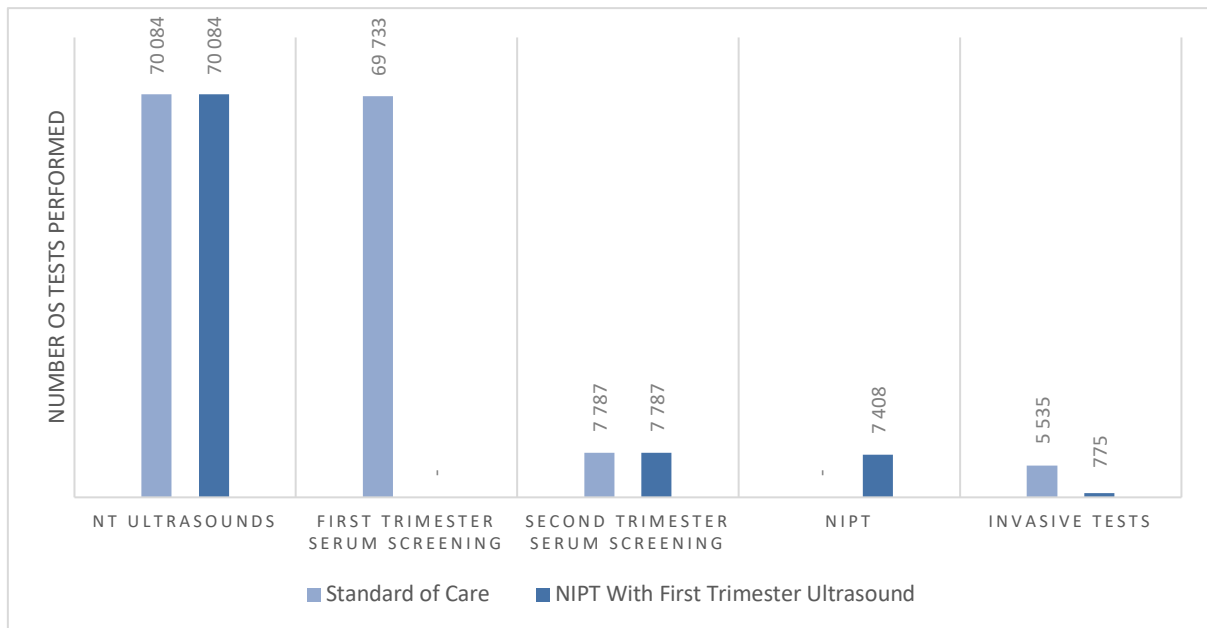


Fig. 8 Number of pregnant women tested in NIPT as contingent screening with first trimester ultrasound (In standard of care, 70,084 first trimester ultrasounds are performed, with 69,733 collecting the biochemical studies to complete the first trimester screening. 7,787 pregnant women are tested with second trimester serum screening. No NIPT tests are performed, and there are 5,535 invasive tests that are completed. In NIPT with first trimester ultrasound, 70,084 first trimester ultrasounds are performed, with no first trimester biochemical screening. There are 7,787 pregnant women who undergo second trimester screening. 7,408 NIPT tests and 775 invasive tests are performed.)

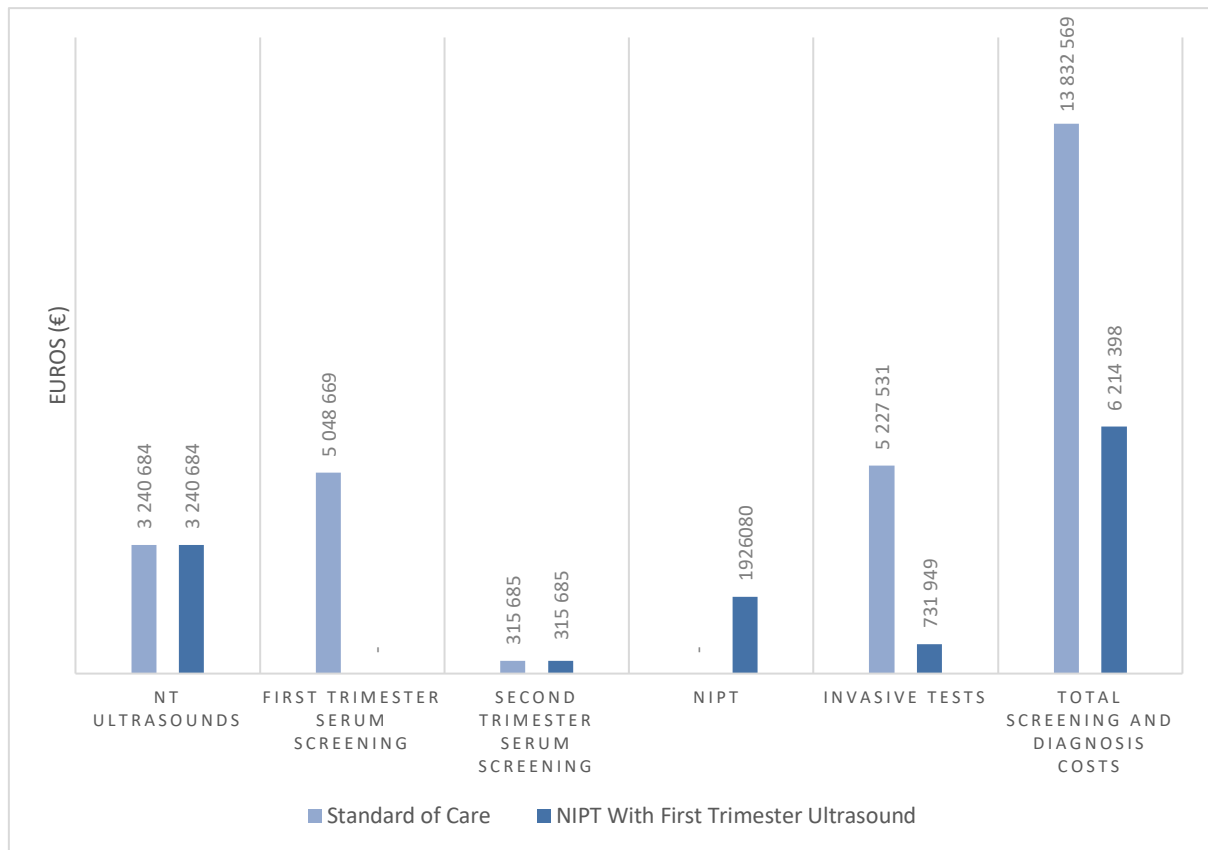


Fig. 9 Testing costs in NIPT as contingent screening with first trimester ultrasound (In standard of care, €3,240,684 is spent on carrying out first trimester ultrasounds, €5,048,669 on the biochemical study to complete the first trimester screening and €315,685 on the second trimester screening. There are no expenses associated with the NIPT but carrying out invasive tests costs €5,227,531. The total screening and diagnosis costs is €13,832,569. Regarding the NIPT with first trimester ultrasound, there is a cost of €3,240,684 for first trimester ultrasounds, without the cost of the first trimester serological biochemical screening. €315,685 is spent on pregnant women who undergo second trimester screening. There is a cost of 1,926,080€ with NIPT and 731,949€ with invasive tests. The total amount for screening and diagnosis is €6,214,398.)

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