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无创产前筛查(NIPT)的"不准确性"与阳性结果的验证*

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摘要：无创产前筛查(Non-Invasive Prenatal Testing, NIPT)通过检测孕妇外周血中的游离胎儿 DNA 来筛查胎儿常见非整倍体，已成为产前筛查中重要的一项技术，甚至可作为高龄孕妇初步筛查的首选方式。但因为难免会出现假阴性和假阳性，所以其阴性结果也并不能总是保证胎儿正常。而对于阳性结果，需通过有创产前诊断进行验证。目前，我国临床主要采用的有创产前诊断方法有绒毛活检(Chorionic Villous Sampling, CVS)、羊膜腔穿刺(Amniocentesis, AC)和脐血穿刺。绒毛活检和羊膜腔穿刺术是 NIPT 阳性结果验证的主要方式。本文主要对造成 NIPT 假阳性和假阴性结果的原因及其阳性结果的验证进行综述。

关键词：无创产前筛查；有创产前诊断；绒毛活检；羊膜腔穿刺

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The Inaccuracy of Non-Invasive Prenatal Testing and the Confirmation of the Positive Results*

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ABSTRACT: Noninvasive prenatal testing (NIPT) has become an important technique in prenatal screening which screen the common aneuploidy through detecting the cell free fetal DNA in the peripheral blood of pregnant woman. It can be the first screening choice of the pregnant woman in advanced age. However, the negative results could not always guarantee the normal fetal and the positive results should be confirmed by the invasive methods because the inevitable false negative and false positive results. At present, the main clinical use of invasive prenatal diagnostic methods are chorionic villous sampling, amniocentesis and umbilical blood sampling, and the first two are the common methods for confirming the positive results. This article mainly reviews the causes of the false negative and the false positive results and the confirmation of the positive results in noninvasive prenatal testing.

Key words: Noninvasive prenatal testing; Invasive prenatal diagnosis; CVS; Amniocentesis

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前言

无创产前筛查(non-invasive prenatal testing，NIPT)通过检测孕妇外周血中游离胎儿 DNA (cell-free fetal DNA，cff-DNA)来筛查胎儿常见非整倍体和性染色体非整倍体，目前已在产前筛查中占据重要地位。2016 年荟萃分析显示 NIPT 对 13 三体、18 三体和 21 三体的灵敏度分别为 97.4 %、97.4 %和 99.3 %，综合特异性为 99.9 %[1]。但是，同任何筛查方法一样，假阳性和假阴性时常发生[2-6]。2015 年的荟萃分析显示对三种常见非整倍体的假阳性率分别为 0.13 %、0.13 %和 0.09 %[7]，阳性预测值分别为 49 %、37 %和 82 %[1]。美国妇产科医师学会和母胎医学学会指出阴性的游离胎儿 DNA 检测结果不能确保胎儿正常，而获得阳性检测结果的孕妇要进行相关的遗传咨询并采取有创产前诊断技术来验证其结果[8]。本文对造成 NIPT 假阳性和假阴性结果的原因及其阳性结果的验证综述如下：

1 影响 NIPT 检测准确性的原因

1.1 染色体嵌合

1.1.1 **限制性胎盘嵌合** (confined placental mosaicism(CPM))限制性胎盘嵌合是指染色体嵌合仅存在于胎盘内而胎儿染色体核型正常的现象[9]，约占 CVS 嵌合的 87 %[10]。二倍体的受精卵在有丝分裂过程中如果发生染色体不分离或三倍体胚胎发生"三体营救"均可造成限制性胎盘嵌合。孕妇血浆中超过 99%的游离胎儿 DNA(cell-free fetal DNA，cff-DNA)来源于胎盘细胞滋养层中的凋亡细胞[11-13]。所以如果胎儿正常，而细胞滋养层存在染色体嵌合且异常细胞系在细胞滋养层中的比例足够高时，往往导致 NIPT 结果出现假阳性。胎盘染色体嵌合可分为三种类型：1 型：染色体异常细胞仅局限于细胞滋养层细胞，

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间充质核心正常。2 型:染色体异常细胞仅出现在间充质核心,细胞滋养层细胞正常。3 型:细胞滋养层细胞和间充质核心均有异常细胞。1 型和 3 型嵌合均可造成 NIPT 假阳性。在报道的 NIPT 阳性病例中,约有 32 %被证实为 CPM[4,14-17]。

1.1.2 **真性胎儿嵌合** (True Fetal Mosaicism,TFM) 上述胎盘嵌合中的后两种类型常常也会累及胎儿[18],即真性胎儿嵌合,约占 CVS 嵌合的 13 %[10]。当细胞滋养层细胞正常,胎儿和间充质核心均存在嵌合时,可导致 NIPT 结果假阴性。当细胞滋养层细胞、间充质核心和胎儿三者均存在嵌合,但是异常细胞系在细胞滋养层中的比例低于一定值,即嵌合水平较低时,NIPT 可能检测不到外周血中异常的 DNA,也会导致假阴性[19]。另外还有一种罕见(发生率约 0.003 %)的嵌合形式即局限性胎儿嵌合(confined fetal mosaicism,CFM),异常染色体仅存在于胎儿,而胎盘染色体正常,也是 NIPT 呈假阴性的原因[20]。

1.2 孕妇细胞污染

研究表明孕妇染色体拷贝数变异(Copy Number Variation,CNV)可导致 NIPT 结果呈假阳性[14,21]。Snyder 研究发现孕妇染色体片段重复可能会增加 NIPT 结果假阳性风险,并据此预测染色体片段缺失可能会造成 NIPT 假阴性。作者通过分析 4 个 NIPT 阳性病例发现其中两个阳性结果是由 18 号染色体大片段重复导致的[22],推测这可能是由于重复的染色体片段释放了较多的 DNA 到母体外周血中而导致的。1997 年至 2016 年发表在 Pubmed 在线数据库中的文献显示由于孕妇 CNV 导致 NIPT 假阳性的病例约为 48 %[14,22]。但也有研究团队对此观点提出质疑,指出 CNV 造成 NIPT 假阳性与使用的技术有关。他们提出采用选择区域数字分析[23]和补偿算法[24,25]可消除 CNV 对 NIPT 假阳性结果的影响。孕妇染色体嵌合也可导致 NIPT 结果呈非整倍体高风险 [26-29]。其中,孕妇 X 染色体嵌合约占 NIPT 性染色体假阳性的 8.6%[29],这可能与随着孕妇年龄增加,其造血细胞系的改变[30]以及卵巢早衰[31,32]均易导致女性 X 染色体嵌合有关。另外,如果孕妇患有涉及染色体异常的恶性肿瘤,也可导致 NIPT 假阳性[33]。Bettegowda 等研究发现在患有转移性实体瘤和局限性癌症的病人中分别有 80 %和 50 %检测到了异常的游离 DNA[34]。Bianchi 研究了 10 个孕妇患有恶性肿瘤的病例,其中有 7 个 NIPT 结果显示多重非整倍体[35]。Snyder 等也研究发现在 37 个 NIPT 结果为多重非整倍体的病例中,有 19 个证实为孕妇患有恶性肿瘤[36]。

1.3 双胎之一死亡

研究显示如果双胞胎中的其中一个胎儿因为染色体异常而死亡,自胎儿死亡后 8 周,来自死亡胎儿的 cff-DNA 仍可在母体外周血中被检测到[37,38]。如果在此期间行 NIPT 检测,就有假阳性的风险,也可导致胎儿性别或 RhD 判断错误[39,40]。除了超声检查可直观地观察到胎儿消失的证据,基于单核苷酸多态性的 NIPT(SNP-NIPT)技术可成功检测出双胎之一死亡[37],可助于减少 NIPT 假阳性发生和防止错误的胎儿性别判断。

1.4 胎儿分数(fetal fraction,ff)

胎儿分数(ff)是 cff-DNA 占孕妇循环血浆中总游离 DNA 的百分比,ff<4%可能导致实验失败或假阴性结果。孕妇体重质量指数(BMI)过高[41]以及 18 三体、13 三体和 X 单倍体胎儿的 ff 降低[42]。如果两个胎儿间的染色体构成不同(如有一个胎儿为非整倍体时),即使总 ff 比单胎增多,但每个胎儿的 ff 是降低的[43]。统计数据显示在至少有一个非整倍体胎儿的多胎妊娠病例中约有 10 %-15 %的病例其 ff<4 %,这样就会有假阴性的风险[44]。

2 NIPT 阳性结果的验证

2.1 常见非整倍体的验证

NIPT 阳性结果的病例应常规行羊膜腔穿刺(Amniocentesis,AC)来验证胎儿染色体核型[45-47]。欧洲人类遗传学协会和美国人类遗传学协会也建议任何 NIPT 阳性结果均应通过有创产前诊断方法验证,首选羊水检测[48]。因为羊水细胞能更加真实的反映胎儿染色体的情况。另外相对 CVS,羊水细胞的染色体嵌合发生率更低[49]。由于我国中孕期血清学筛查比较普及,所以中孕期羊水检测可能是 NIPT 阳性结果确认的主要方式。但有学者认为由于 AC 的最佳检测时间为 15-22 周,而 NIPT 可最早在第 10 周时进行,对于结果阳性的孕妇,等待时间较长,可能不是一种可被接受的方式[50]。另外,如果羊水培养发现胎儿异常,再行终止妊娠可能时间较晚。而 CVS 可在 10-14 周取样,可较早发现异常胎儿并及时进行干预,避免了孕妇等待的焦虑。另有文献指出从长期经验来看,如果使用恰当的操作程序,利用 CVS 细胞遗传学分析来筛查胎儿 13 三体、18 三体和 21 三体是比较可靠的[51]。

CVS 经腹、阴道或宫颈对胎盘绒毛进行取样,主要有三种成分:外层的合胞体滋养层,中间的细胞滋养层和内层的间充质核心。CVS 主要分析的细胞成分是后两种,分别通过直接法(或短时培养法 (short-term culture,STC)) 和长期培养法(long-term culture,LTC)进行分析[52,53]。因为细胞滋养层细胞的染色体核型并不能完全代表胎儿的染色体组成[54],所以单独的 STC 法不建议用于产前诊断(ACC Prenatal Diagnosis Best Practice Guidelines 2009)。Grati 通过回顾 52673 份绒毛的细胞滋养层细胞和间充质核心的细胞遗传学结果,发现对于常见非整倍体染色体和性染色体,当细胞滋养层或间充质核心其中之一显示嵌合时,胎儿要么为真性嵌合要么正常。而如果细胞滋养层和间充质核心均显示非整倍体时,胎儿通常也是非整倍体[55]。根据此结论,有评论指出 CVS 作为 NIPT 阳性结果的验证方式是可靠的,但需对 CVS 中的所有细胞系进行分析,即用 FISH (fluorescence in situ hybridization) 或短时培养对未培养的细胞滋养层进行分析,同时用长期培养法对间充质核心进行分析。若两种方法的结果均为非整倍体,可报告结果。若其中之一为嵌合,则需行 AC 以鉴别 CPM 和 TFM,AC 需同时采用 FISH 和染色体核型分析两种方法 [18]。但需注意的是低水平的 TFM 可能致羊水细胞培养阴性,遗传咨询时应告知孕妇及家属[55]。所以当 NIPT 结果异常,但羊水检测结果正常时,即使新生儿表型正常,也应在分娩时对胎盘和新生儿外周血进行染色体核型分析以及时发现胎儿体细胞低水平嵌合,以便监测嵌合所致的进行性的发育综合征[4]。

2.2 性染色体非整倍体的验证

如果 NIPT 结果提示性染色体非整倍体高风险,应直接采用 AC 方法验证。一方面因为 CVS 对于胎儿性染色体异常的预测值较低,CVS 检测结果中的 45,X 被证明对于胎儿的核型预测是无意义且不稳定的指标[56,57]。另一方面因为 NIPT 检测中

性染色体非整倍体高风险相对高的发生率多是因为母体存在性染色体嵌合[29]。在所有的 NIPT 性染色体阳性结果中,应该考虑到是否由于孕妇存在性染色体嵌合而导致的,但同时也要认识到即使证实了 NIPT 性染色体非整倍体确实由于母体异常细胞污染导致,也并不能排除胎儿本身也是性染色体非整倍体的可能性。所以,对于 NIPT 性染色体的非整倍体验证,羊水检测应为首选的方式。

2.3 QF-PCR 有助于检出单亲二倍体(Uniparental Disomy,UPD)

有文献报道了一例 22 个月大的女孩有严重发育迟缓、先天大脑发育不良和先天性心脏病,证实是因为父亲减数分裂染色体不分离导致 " 三体营救 " 而发生了 9 号染色体的单亲二倍体和三倍体细胞的嵌合。FISH 检测体细胞 T9 嵌合水平为 20 %,或因嵌合水平较低而导致前期羊水细胞检查结果正常(NIPT 结果为 T9 阳性)[58]。这个病例提醒我们当 NIPT 提示三倍体高风险,而羊水检查结果阴性时,一方面要考虑低水平嵌合,另一方面也要考虑 UPD 存在的可能,尤其涉及到印记染色体(6, 7, 11, 14, 15, 20)时,更应提高警惕。QF-PCR 可以有助于检出 UPD,有作者提出在使用传统的有创产前诊断方法之外,有必要利用短串联重复序列标记的 QF-PCR 方法检测 UPD 的存在[59]。对于类似病例,还应该严密关注孕期超声检查,如果超声提示有胎儿宫内发育迟缓或其他可鉴别的异常结构,均应引起重视并及时采取适当的干预措施,防止异常胎儿的出生。

3 总结

NIPT 的出现大大减少了很多不必要的有创检查,降低了流产率和感染率,为孕妇减轻了焦虑,也为妇产科医生减轻了负担。但是造成 NIPT 假阳性和假阴性结果的生物学因素是一直存在的,并且目前阶段并不能在技术层面上消除这些因素的影响,所以其 " 不准确 " 性也是不可避免的。所有阳性结果均需通过 CVS 或 AC 方法进行验证。NIPT 结果异常但羊水培养阴性的病例可能还需要对胎盘或新生儿外周血进行 FISH 或染色体核型分析。QF-PCR 和 SNP-NIPT 等方法有助于 UPD 和双胎之一死亡病例的检出。NIPT 结果阴性病例要重视结构超声的检查,一旦发现异常应立即行有创诊断。在临床实践中,妇产科医生可能需要根据孕周、非整倍体类型以及孕妇自身情况等制定个体化的产前筛查和诊断策略,并对各项检查结果进行全面而专业的评价,最终做出最接近胎儿真实状况的判断。

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