P-73

Exploring PPV by indication for screen-positive CNVs on genome-wide cfDNA

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1. Introduction

Genome-wide cfDNA (GW-cfDNA) allows screening for a broader spectrum of chromosome abnormalities in pregnancy, including genome-wide copy number variants (CNVs). Recently, a retrospective review[1] of >85,000 GW-cfDNA samples at one commercial laboratory found that the positivity rate for genome-wide CNVs (not including isolated select microdeletion syndromes <7 Mb) was 0.56% (n=490). Diagnostic testing outcomes were available in 50% of cases (n=244) with an overall positive predictive value (PPV) of 74.2% in those cases. Here, we explore whether PPV varied by the indication for the GW-cfDNA screen.

2. Methods

A retrospective review of >85,000 GW-cfDNA samples from singleton pregnancies submitted for screening was queried to explore the relationship between testing indication and PPV. Samples had been subjected to DNA extraction, library preparation, and whole genome massively parallel sequencing.[2,3] Sequencing data were analyzed using a proprietary algorithm to detect trisomies and subchromosomal genome-wide CNVs 7 Mb and larger.[3] Screen positive cases with a CNV were reviewed and diagnostic testing outcomes were collected as described in Rafalko, 2021 and assigned as either 'true positive' or 'false positive.' Indications for testing were provided on the test requisition form by the ordering clinician. No known high-risk indication includes cases sent for routine/average risk screening, as well as cases in which a testing indication was not specified and maternal age was less than 35 years old on the test requisition form.

3. Results

Table 1 shows a breakdown of PPV by indication, with the total number of positives, as well as true and false positives, for each indication. **Figure 1** illustrates the PPVs (the last column of Table 1) as a bar graph. PPVs were highest in cases with a personal/family history, ultrasound findings (USF), or an abnormal maternal serum screen. However, the latter has fewer cases compared to the other groups. PPVs were lower for cases tested due to advanced maternal age.

Additionally, cases with multiple indications were examined in order to identify whether USF was one of the listed indications, and if the presence of USF would impact PPV. There were 22 cases of 'multiple indications' that included USF as one of the indications (19 true positives and 3 false positives). The rest of the cases were presumed to be without USF for the purposes of this calculation. The PPV for cases with USF was 87.7%, while the PPV for cases (presumably) without USF was 62.3%, as illustrated by **Figure 2**.

4. Conclusions

As expected, the PPV for CNVs is higher in cases with ultrasound findings compared to cases without. However, the PPV for cases without ultrasound findings was >60%, suggesting that these findings were still more likely to be true positives than false positives. Additionally, cases referred for a personal or family history of a chromosome abnormality had high PPVs, likely because many of these families were known carriers of a chromosome rearrangement.[1] Lastly, cases with abnormal maternal serum screening as the indication had higher PPVs, but this group had less than 10 total cases in the cohort, which limits interpretation of this result. A larger cohort would allow for further analysis of the relationship between PPV for CNVs and this indication.

This data may be useful for providers counseling patients with a positive GW-cfDNA result for a CNV, especially in context of an anomaly on ultrasound. As always, diagnostic testing is recommended for any patient with a positive cfDNA result. Diagnostic testing with microarray would provide the patient with confirmation of the presence (or absence) of the CNV found by genome-wide cfDNA in the fetus and may also detect additional chromosome anomalies outside of the scope of genome-wide cfDNA. As discussed in Rafalko et al, many of the false positive cases (63%) had probable biological explanations for the discordant results (such as fibroids, UPD, or maternal fragile sites), which may be associated with clinical implications for the fetus and/or the pregnant person.

The data presented here is limited by a small sample size for some indications and relies on accuracy of testing indication information as provided on the test requisition forms. A larger cohort of patients would allow for a more robust analysis of these findings.

Tables + Figures

Table 1: PPV by genome-wide cfDNA indication

	PPV by indications			
	True positives	False positives	Total positives	PPV
No known high-risk indication	20	6	26	76.9%
Maternal age	34	36	70	48.6%
Ultrasound findings	81	11	92	88.0%
Personal or family history	17	2	19	89.5%
Multiple indications	21	6	27	77.8%
Abnormal maternal serum screen	7	1	8	87.5%
Other	1	1	2	50.0%
Totals	**181**	**63**	**244**	**74.2%**

Figure 1: PPV by indication for testing

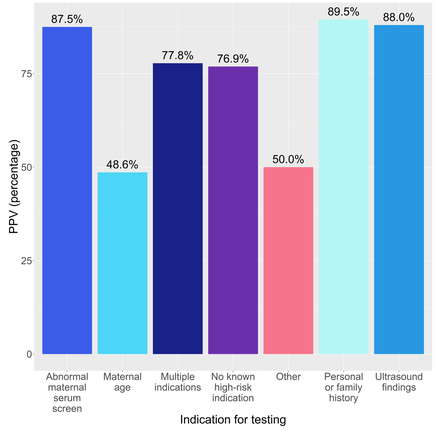
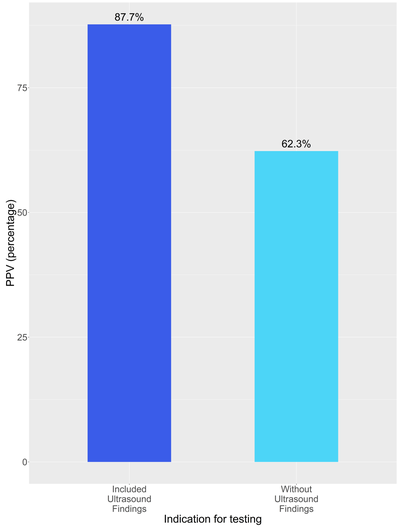


Figure 2: Comparison of PPV for cases with and without ultrasound findings



References

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