

Supplementary Material

Prenatal cell-free DNA testing of women with pregnancy-associated cancer: a retrospective cross-sectional study

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Supplemental Table 1: The “Maastricht criteria” to interpret NIPT results for suspicion of a maternal malignancy (1).

Criteria	Score
Number of aberrations ^a >2	3
Monosomy	2
Segmental aneuploidies with presence of oncogene or tumour suppressor-gene	2
Trisomy 12	2
Trisomy 9	2
Trisomy 8	1
Two trisomies (in total)	1
High significance score of aberration ^{a,b}	1
Malignancy suspicious-NIPT	Total score
No suspicion	<2
Mild suspicion	2
Strong suspicion	≥3
<p>Multiple criteria may apply to one NIPT result, individual scores are then counted together. ^a Aberration: any type of aberration, i.e. partial duplications (gains), partial deletions (losses), trisomy, monosomy. ^b High significance score based on the number of standard deviations from the mean value of the reference, dependent of the algorithm used. For example a Stouffer z-score of segmental aneuploidy or a t-statistic of aneuploidy ≤ -10 or $\geq +10$.</p>	

Supplemental Table 2: NIPT results of all patients with tumour type and stage (n=65)

Study number	Tumour type	Tumour stage at diagnosis	Time between NIPT and diagnosis (days)	Genome-wide / targeted NIPT	Initial NIPT result	Reviewed NIPT result# - "Maastricht criteria" score	Details reviewed NIPT, "Maastricht criteria" score
Breast cancer (n=20)	<u>0</u>						
NIPT-09	Breast cancer, Hormone positive, HER2 negative	Stage I	31	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-14	Breast cancer, Triple negative	Stage I	47	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-20	Breast cancer, Triple negative	Stage II	101	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-21	Breast cancer, Hormone positive, HER2 negative	Stage II	189	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-26	Breast cancer, Triple negative	Stage II	27	GW	Suspicious of a malignancy	Score 5: Strong suspicion of malignancy	Number of alterations: 5 (part)dup10p (<i>CCNY</i> gene, <i>MAP3K8</i> gene)
NIPT-32	Breast cancer, Triple negative	Stage II	43	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-35	Breast cancer, Triple negative	Stage II	2	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-36	Breast cancer, Triple negative	Stage II	94	Targeted	Non-suspicious	Score 0: No suspicion	N/A
NIPT-37	Breast cancer, Hormone positive, HER2 negative	Stage II	95	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-39	Breast cancer, Hormone positive, HER2 negative	Stage II	96	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-40	Breast cancer, Triple negative	Stage II	69	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-41	Breast cancer, Triple negative	Stage II	117	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-47	Breast cancer	Stage II	104	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-56	Breast cancer, Hormone positive, HER2 positive	Stage II	6	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-10	Breast cancer, Hormone positive, HER2 negative	Stage III	78	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-16	Breast cancer, Hormone positive, HER2 positive	Stage III	73	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-54	Breast cancer, Triple negative	Stage III	174	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-34*	Breast cancer, Triple negative	Stage IV	22	GW	Suspicious of a malignancy	Score 6 Strong suspicion of malignancy	Number of alterations: 15 M18 (part)dup13q (z-score 15,9)

NIPT-19	Breast cancer, Triple negative	Stage IV	175	Targeted	Non-suspicious	Score 0: No suspicion	N/A
NIPT-44	Breast cancer, Hormone negative, HER2 positive	Stage IV	57	Targeted	Non-suspicious	Score 0: No suspicion	N/A
Gynaecological cancer (n=19)							
NIPT-01	Cervical cancer, squamous cell carcinoma	Stage I	118	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-02	Cervical cancer, adenocarcinoma	Stage I	145	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-03	Cervical cancer, squamous cell carcinoma	Stage I	115	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-05	Cervical cancer squamous cell carcinoma	Stage I	0	Targeted	Non-suspicious	Score 0: No suspicion	N/A
NIPT-24	Cervical cancer, squamous cell carcinoma	Stage I	148	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-33	Cervical cancer, squamous cell carcinoma	Stage I	97	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-42	Cervical cancer, squamous cell carcinoma	Stage I	22	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-59	Cervical cancer, adenocarcinoma	Stage I	104	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-63	Cervical cancer, squamous cell carcinoma	Stage I	212**	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-66	Cervical cancer, adenocarcinoma	Stage I	165	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-53	Cervical cancer, squamous cell carcinoma	Stage II	66	Targeted	Non-suspicious	Score 0: No suspicion	N/A
NIPT-60	Cervical cancer, adenocarcinoma	Stage II	35	Targeted	Non-suspicious	Score 0: No suspicion	N/A
NIPT-65	Cervical cancer, squamous cell carcinoma	Stage II	11	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-67	Cervical cancer, adenocarcinoma	Stage II	187**	GW	Non-suspicious	Score 0: No suspicion	Possible related CNV but below lower limit of windowed bin test: 3q26 gain detected (7Mb), <i>MECOM</i> , <i>PRKCI</i> , <i>TERC</i> genes, associated with cervical cancer (2)
NIPT-50	Cervical cancer, squamous cell carcinoma	Stage III	100	GW	Aberrant, other than suspicious of a malignancy (structural chromosomal aberration)	Score: 2 Mild suspicion of malignancy	dup9p (<i>JAK2</i> , <i>CD274</i> , <i>PDCD1LG2</i> genes)
NIPT-61	Cervical cancer, squamous cell carcinoma	Stage III	402**	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-45	Ovarian cancer, high grade serous epithelial	Stage I	19	Targeted	Non-suspicious	Score 0: No suspicion	N/A
NIPT-43	Ovarian cancer, high grade serous epithelial	Stage III	25	GW	Suspicious of a malignancy	Score: 8 Strong suspicion of malignancy	Number of alterations: 28 M13, M21, M22

							Recurrent CNVs in ovarian cancer and this NIPT: (part)dup1q, (part) dup3q, (part)dup8q, (part)dup20q, del22, del6q (3). (part)dup8q (z-score 10,5)
NIPT-04	Vulvar cancer, squamous cell carcinoma	Stage III	161	Targeted	Non-suspicious	Score 0: No suspicion	N/A
Haematological cancer (n=11)							
NIPT-31*	Hodgkin lymphoma	Stage I	47	GW	Suspicious of a malignancy	Score: 5 Strong suspicion of malignancy	Number of alterations: 9 dup2p(<i>REL</i> gene), dup5p, del6q
NIPT-55	Hodgkin lymphoma	Stage II	29	GW	Suspicious of a malignancy	Score: 7 Strong suspicion of malignancy	Number of alterations: 8 M11, M13 T5, dup9p (<i>JAK2</i> , <i>CD274</i> , <i>PDCD1LG2</i> genes)
NIPT-58*	Hodgkin lymphoma	Stage II	38	GW	Suspicious of a malignancy	Score: 8 Strong suspicion of malignancy	Number of alterations: 13 M7, M13 dup2p(<i>REL</i> gene), del6q (<i>TNFAIP3</i> gene) T5, T15, T18, T20 dup2p (z-score 13,0)
NIPT-64	Hodgkin lymphoma	Stage II	29	GW	Suspicious of a malignancy	Score: 5 Strong suspicion of malignancy	Number of alterations: 7 dup5p, dup9p (<i>JAK2</i> , <i>CD274</i> , <i>PDCD1LG2</i> genes)
NIPT-46*	Hodgkin lymphoma	Stage III	148	GW	Suspicious of a malignancy	Score: 7 Strong suspicion of malignancy	Number of alterations: 16 M6, M15 dup2p(<i>REL</i> gene), dup5p, dup9p, (<i>JAK2</i> , <i>CD274</i> , <i>PDCD1LG2</i> genes) dup9p (z-score 16,7), (part)dup22q (z-score 11,9)
NIPT-11	Hodgkin lymphoma	Stage IV	57	GW	Suspicious of a malignancy	Score: 7 Strong suspicion of malignancy	Number of alterations: 9 M4 dup2p(<i>REL</i> gene), dup5p, dup9p, (<i>JAK2</i> , <i>CD274</i> , <i>PDCD1LG2</i> genes)
NIPT-28*	Non Hodgkin lymphoma, primary mediastinal B-cell lymphoma	Stage I	69	GW	Suspicious of a malignancy	Score: 12 Strong suspicion of malignancy	Number of alterations: 8 M15, M16 Chr 9 (<i>JAK2</i> , <i>CD274</i> , <i>PDCD1LG2</i> genes) T9, T12, T20
NIPT-30*	Non Hodgkin lymphoma, primary mediastinal B-cell lymphoma	Stage I	41	GW	Suspicious of a malignancy	Score: 8 Strong suspicion of malignancy	Number of alterations: 17 (part)dup2p(<i>REL</i> gene), T9 (<i>JAK2</i> , <i>CD274</i> , <i>PDCD1LG2</i> genes) T9 (part)dup2p(z-score 9.2), (part)dup3p(z-score 14.0), (part)dup8q(z-score 15.6), dup18q z-score 10.5)
NIPT-57	Non Hodgkin lymphoma, diffuse large B-cell lymphoma	Stage IV	56	GW	Suspicious of a malignancy	Score: 5 Strong suspicion of malignancy	Number of alterations: 6 Recurrent CNVs seen in DLBCL and this NIPT: dup6p, dup7q, dup11q, dup18q, del6q (4). Chr7 (z-score chr wide 22)
NIPT-62	Non Hodgkin, diffuse large B-cell lymphoma	Stage IV	193**	Targeted	Non-suspicious	Score 0: No suspicion	N/A
NIPT-48	Lymphoma grey zone (Hodgkin and diffuse large B-cell lymphoma)	Stage III	144	GW	Aberrant, other than suspicious of a malignancy (structural chromosomal aberration)	Score: 3 Strong suspicion of malignancy	dup5p, dup9p (<i>JAK2</i> , <i>CD274</i> , <i>PDCD1LG2</i> genes) chr9 (z-score 11,4)

Melanoma (n=6)							
NIPT-07	Melanoma	Stage I	120	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-12	Melanoma	Stage I	140	Targeted	Non-suspicious	Score 0: No suspicion	N/A
NIPT-25	Melanoma	Stage I	150	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-51	Melanoma	Stage II	155	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-49	Melanoma	Stage III	73	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-18	MELTUMP (melanocytic tumour of unknown malignant potential), Naevoid melanoma	Unknown	Exact date of recurrence unknown (after NIPT)	Targeted	Non-suspicious	Score 0: No suspicion	N/A
Nasopharyngeal cancer (n=2)							
NIPT-52	Nasopharynx tumour, non-keratinizing	Stage II	71	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-29	Nasopharynx tumour, sino nasal undifferentiated carcinoma	Stage IV	154	GW	Non-suspicious	Score 0: No suspicion	N/A
Thyroid cancer (n=2)							
NIPT-17	Thyroid cancer, papillary subtype	Stage III	0	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-22	Thyroid cancer, papillary subtype	Unknown	0	GW	Non-suspicious	Score 0: No suspicion	N/A
Other (n=5)							
NIPT-15*	Colorectal carcinoma, MSS	Stage IV	58	GW	Suspicious of a malignancy	Score: 9 Strong suspicion of malignancy	Number of alterations: 12 M4, M9, M14 CNVs frequently seen in MSS CRC and this NIPT; T7, T13, dup20q, del18p, del18q (5). T2, T13, T20
NIPT-38*	CUP midline with lymph node metastasis liver hilus	N/A	52	Targeted	Suspicious of a malignancy	Score: 2 Mild suspicion	(prox)del13q, 27Mb
NIPT-13	Thymoma type B1	Stage IV	11	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-68	Bladder cancer, urothelial carcinoma	Stage I	71	GW	Non-suspicious	Score 0: No suspicion	N/A
NIPT-06	Meningioma	Stage II	138	GW	Non-suspicious	Score 0: No suspicion	N/A

#Reviewed according to the “Maastricht criteria”. *Patients also described in study population of our previous study (6). **Postpartum diagnosis of cancer.

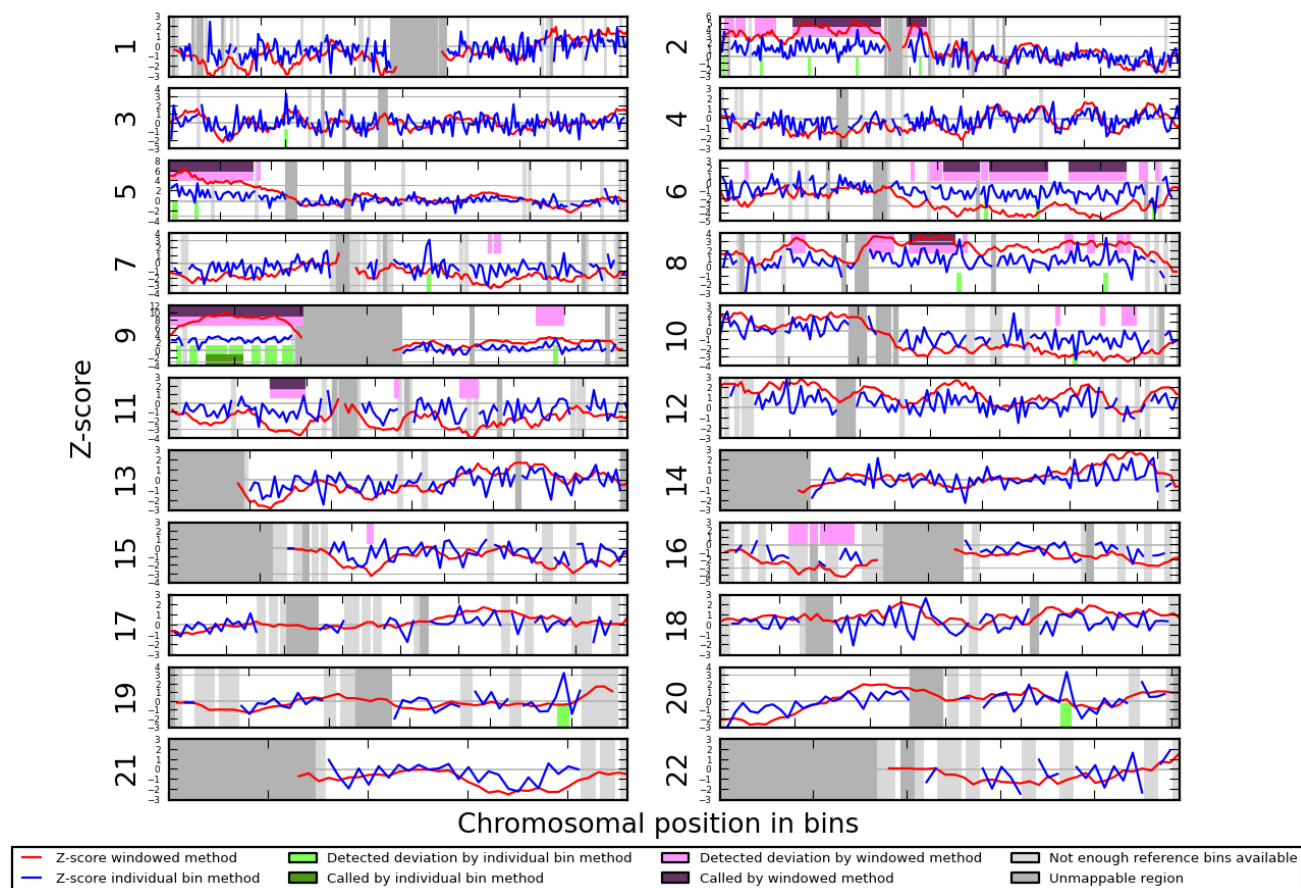
Abbreviations: Chr; chromosome, CNV; copy number variation, CRC; colorectal cancer, CUP; carcinoma of unknown primary, del; deletion, dup; duplication, GW; genome-wide, M; monosomy, MSS, microsatellite stable, N/A; not applicable, prox; proximal, T; trisomy.

Supplemental Table 3: Symptoms of disease at diagnosis (n=65)

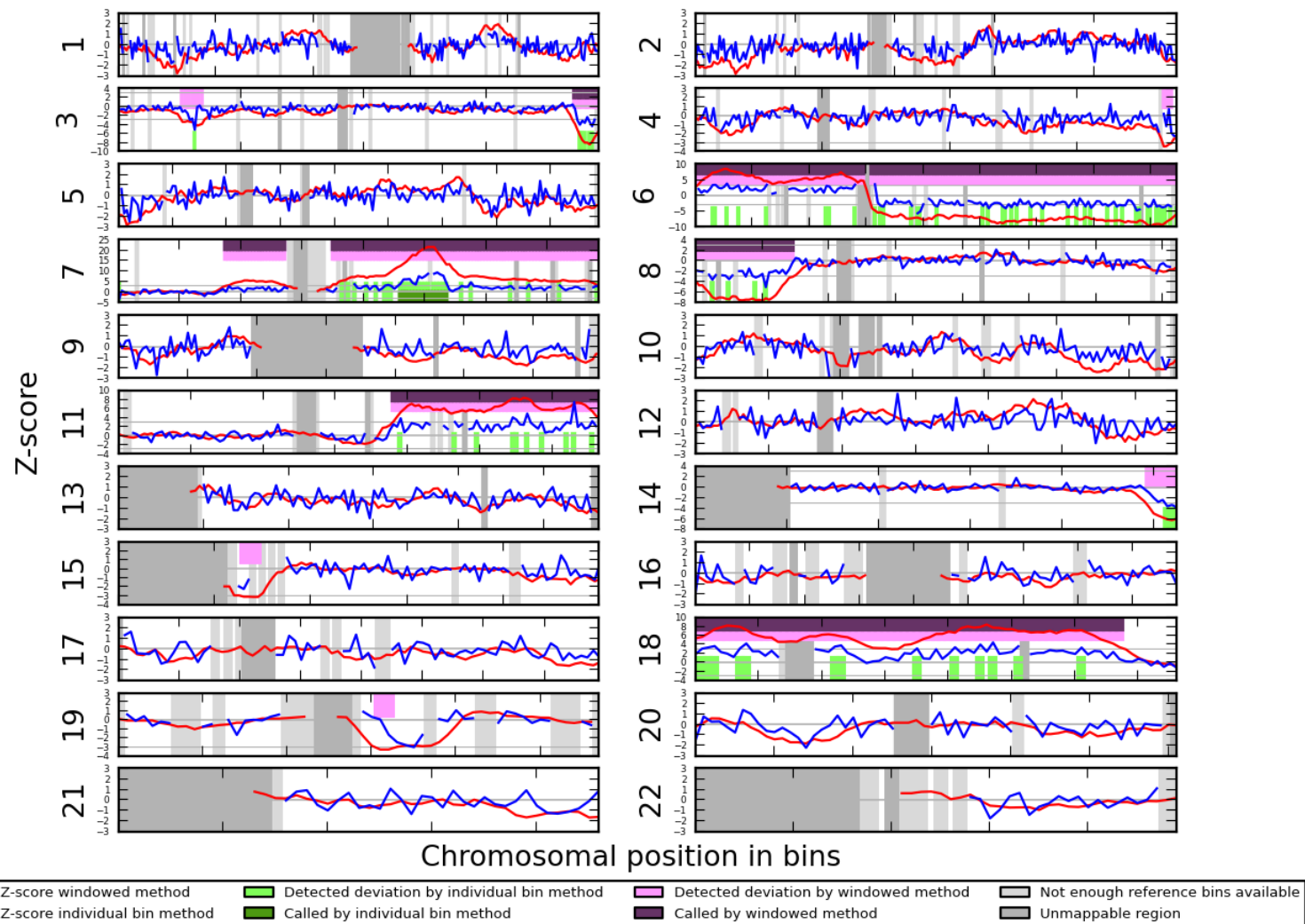
Malignancy suspicious-NIPT*	Patients n=16
Symptoms	6
Lymphadenopathy at physical examination	3
Coughing, weight loss, less appetite	1
Sore pelvis, lower back pain	1
Abnormal vaginal bleeding/discharge	1
Asymptomatic	7
Unknown	3
Non-suspicious NIPT	Patients n=49
Symptoms	42
Abnormal vaginal bleeding/discharge and/or abnormal PAP smear	15
Palpable mass/lesion/lymphadenopathy at physical examination	20
Dyspnoea	1
Epistaxis	1
Haematuria	1
Intracerebral bleeding left frontal	1
Unknown	3
Asymptomatic	1
Unknown	6

*Strong suspicion of a maternal malignancy or mild suspicious of a maternal malignancy.

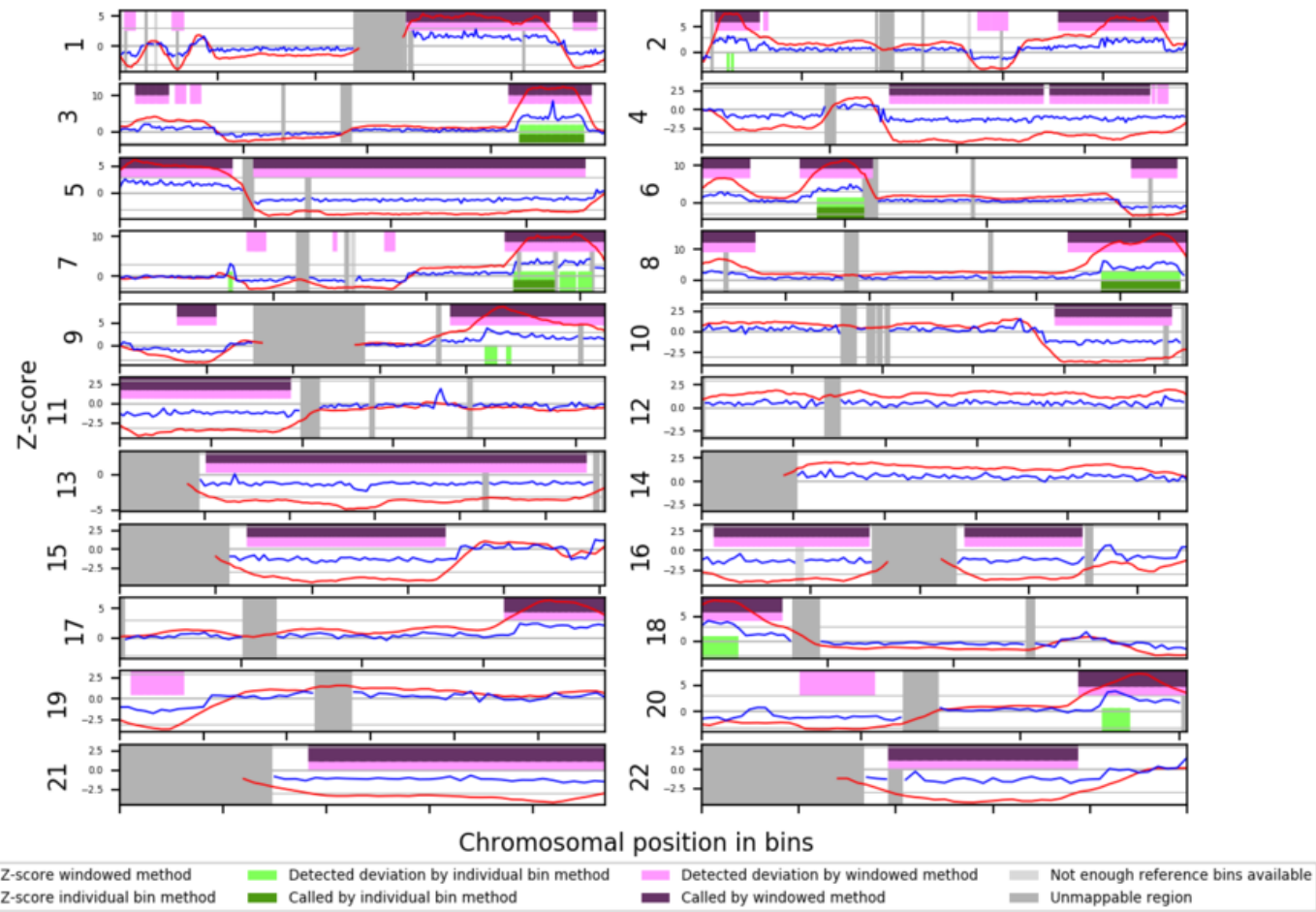
Supplemental Figure 1: Highlighted WISECONDOR NIPT results: All 22 autosomal chromosomes are displayed. For each chromosome, the vertical axis depicts the z-score, and the horizontal axis from left to right the 1 Mb-bins from p- to q-telomere. The vertical and horizontal axes are min-max normalized to a uniform height and width, respectively. The blue line represents the z-score per individual bin, the red line is the z-score using the windowed bin method (7). Dark purple bars represent called regions of at least 10 consecutive deviated bins by the windowed bin method. Dark green bars represent regions of at least 10 consecutive deviated bins called by the individual bin method. The light purple and light green bins have deviated z-scores by, respectively, the windowed bin or the individual bin method.



Supplemental figure 1a: Genome-wide NIPT analysis of a patient with a classic Hodgkin lymphoma, stage II (NIPT-64), with typical copy number variations that are frequently seen in patients with classic Hodgkin lymphoma (6).



Supplemental figure 1b: Genome-wide NIPT analysis of a patient with a Non-Hodgkin lymphoma, diffuse large B-Cell lymphoma, stage IV (NIPT-57), with typical copy number variations that are frequently seen in patients with diffuse large B-cell lymphoma (4).



Supplemental figure 1c: Genome-wide NIPT analysis of a patient with ovarian cancer, stage III (NIPT-43), with typical copy number variations that are frequently seen in patients with ovarian cancer (3).

Supplemental reference list

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3. Integrated genomic analyses of ovarian carcinoma. *Nature*. 2011;474(7353):609-15.
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