Supplementary Material

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**Supplemental Methods**

To enable method comparison, spot urine samples of 39 neuroblastoma and non-neuroblastoma patients (i.e., patients with other paediatric cancer types or no malignancy) from the Amsterdam UMC and Princess Maxima Centre were analysed by both HPLC-FD and UPLC-MS/MS (Table S1). Urine samples were collected at diagnosis and during therapy. At the Amsterdam UMC, urine samples were protected from light, acidified using hydrochloric acid and stored at -25°C. At the Princess Maxima Centre, urine samples were protected from light without prior acidification and stored at -20°C. All 39 samples were analysed in one batch by HPLC-FD and UPLC-MS/MS as previously described.1,2

**Supplementary Table 1 Cohort overview of patients used for method comparison**

|  |  |  |
| --- | --- | --- |
| **Center** | **Diagnosis and time of urine collection** | **N** |
| **Princess Maxima Center** | Neuroblastoma, at diagnosis | 2 |
| **Amsterdam UMC** | Neuroblastoma, follow-up | 26 |
| **Amsterdam UMC** | Nephroblastoma | 2 |
| **Amsterdam UMC** | Pheochromocytoma | 1 |
| **Amsterdam UMC** | Ganglioneuroma | 1 |
| **Amsterdam UMC** | Rhabdoïd tumor | 1 |
| **Amsterdam UMC** | Adrenocortical carcinoma | 1 |
| **Amsterdam UMC** | No malignancy | 5 |
| **Total** |  | 39 |

**Amsterdam UMC, Amsterdam University Medical Centre.**

**Supplementary Table 1** Reference values of urinary catecholamines metabolites for each age-group (days) tested by UPLC-MS/MS

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **UPLC-MS/MS** | |  | | | | | | | | |
| **Age (days)** | **N** | | **HVA1** | **VMA1** | **DA2** | **3MT2** | **NE2** | **NMN2** | **E2** | **MN2** |
| **0 – 180** | 95 | | 9 – 25 | 4 – 17 | 161 – 1527 | 40 – 278 | 12 – 160 | 20 – 144 | 0 – 17 | 3 – 34 |
| **181 – 360** | 82 | | 8 – 22 | 6 – 15.1 | 261 – 1294 | 68 – 173 | 16 – 133 | 23 – 92 | 1 – 22 | 12 – 46 |
| **361 – 730** | 80 | | 8 – 20 | 5 – 13 | 198 – 1170 | 48 – 144 | 27 – 195 | 22 – 84 | 1 – 22 | 14 – 54 |
| **731 – 2190** | 97 | | 5 – 15 | 3 – 10 | 207 – 1031 | 27 – 114 | 19 – 99 | 16 – 55 | 1 – 23 | 15 – 54 |
| **2191 – 3650** | 85 | | 3 – 10 | 3 – 7 | 127 – 533 | 20 – 63 | 13 – 91 | 10 – 47 | 1 – 21 | 14 – 43 |
| **3651 – 5840** | 88 | | 2 – 7 | 1 – 5 | 166 – 431 | 19 – 57 | 14 – 64 | 7 – 31 | 1 – 18 | 11 – 37 |
| **5841– 43800** | 44 | | 1 – 7 | 1 – 5 | 81 – 253 | 11 – 29 | 15 – 66 | 7 – 25 | 1 – 19 | 4 – 22 |

The reference values were constructed based on the 5th–95th percentiles. 1HVA and VMA concentrations are reported in mmol/mol creatinine, 2Catecholamines and metanephrines concentrations in µmol/mol creatinine. 3MT, 3-methoxytyramine; DA, dopamine; E, epinephrine; HPLC-FD, High Performance Liquid Chromatography coupled with fluorescence detection; HVA, homovanillic acid; MN, metanephrine; NE, norepinephrine; NMN, normetanephrine; VMA, vanillylmandelic acid

**Supplementary Table 2** Patient characteristics in various urine sample groups

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | **HPLC-FD Successful 24h collection (n = 171)** | **HPLC-FD Failed 24h collection  (n = 63)** | **HPLC-FD Spot**  **(n = 62)** | **UPLC-MS/MS Spot**  **(n = 104)** |
| **Age** | Median in months | 22.8 | 26.6 | 24.8 | 24.3 |
|  | <18 months (%) | 81 (47) | 24 (38) | 25 (40) | 46 (44) |
|  | ≥18 months (%) | 90 (53) | 39 (62) | 37 (60) | 58 (56) |
| **Gender** | Male (%) | 107 (63) | 29 (46) | 32 (52) | 52 (50) |
|  | Female (%) | 64 (37) | 34 (54) | 30 (48) | 52 (50) |
| **Stage** | Other than 4 (%) | 92 (54) | 22 (35) | 30 (48) | 36 (35) |
|  | 4 (%) | 79 (46) | 41 (65) | 32 (52) | 68 (65) |
| **Morphology** | Neuroblastoma (%) | 141 (83) | 55 (87) | 54 (87) | 95 (91) |
|  | Ganglioneuroblastoma (%) | 30 (17) | 8 (13) | 8 (13) | 9 (9) |
| ***MYCN*** | Not amplified (%) | 129 (75) | 47 (75) | 46 (74) | 78 (75) |
|  | Amplified (%) | 38 (22) | 14 (22) | 12 (19) | 20 (19) |
|  | Unknown (%) | 4 (3) | 2 (3) | 4 (7) | 6 (6) |

Neuroblastoma patients were grouped based on the mode of urine collection and analysis method. 296 patients from this cohort have been published previously. 24h, 24-hour urine collection. HPLC-FD, High Performance Liquid Chromatography coupled with fluorescence detection; UPLC-MS/MS, ultra-performance liquid chromatography coupled with electrospray tandem mass spectrometry

**Supplementary Table 3** Comparison of patient characteristics between urine sample groups

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | **HPLC-FD**  **Failed 24h vs Successful 24h**  **P-values** | **HPLC-FD Successful 24h vs spot**  **P-values** | | **Spot**  **HPLC-FD vs UPLC-MS/MS**  **P-values** |
| **Age** | <18 months | 0.21 | 0.34 | 0.62 | |
|  | ≥18 months |
| **Gender** | Male | 0.02\* | 0.13 | 0.84 | |
|  | Female |
| **Stage** | Other than 4 | 0.01\* | 0.46 | 0.08 | |
|  | 4 |
| **Morphology** | Neuroblastoma | 0.37 | 0.4 | 0.51 | |
|  | Ganglioneuroblastoma |
| ***MYCN*** | Not amplified | 0.94 | 0.3 | 0.98 | |
|  | Amplified |
|  | Unknown |

Statistical significance was evaluated with chi-square test. P < 0.05 is regarded as statistically significant. For exact patient number in each group see Supplementary Table 1. 24h, 24-hour urine collection; HPLC-FD, High Performance Liquid Chromatography coupled with fluorescence detection; UPLC-MS/MS, ultra-performance liquid chromatography coupled with electrospray tandem mass spectrometry

**Supplementary Figure 1** Mode of urine collection and urinary catecholamine metabolite in neuroblastoma patients

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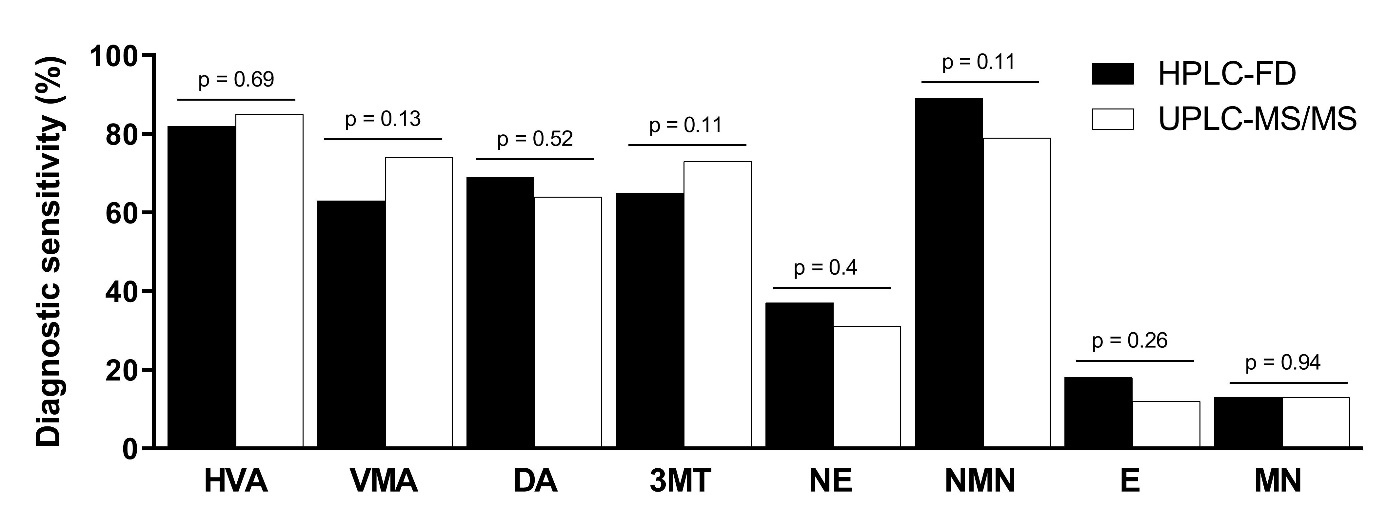
Urine was collected at diagnosis as spot urine (Spot, n = 62) or as successful 24-hour collection (24h, n = 171) and analysed by HPLC-FD. Metabolite concentration were normalised to creatinine concentration. Closed box with line represents median with 25th and 75th percentile and bars represent 2.5th and 97.5th percentile. A = homovanillic acid (HVA), B = vanillylmandelic acid (VMA), C = dopamine (DA), D = 3-methoxytyramine (3MT), E = norepinephrine (NE), F = normetanephrine (NMN), G = epinephrine (E), H = metanephrine (MN).

**Supplementary Figure 2** Comparison of urine samples analysed by both HPLC-FD and UPLC-MS/MS



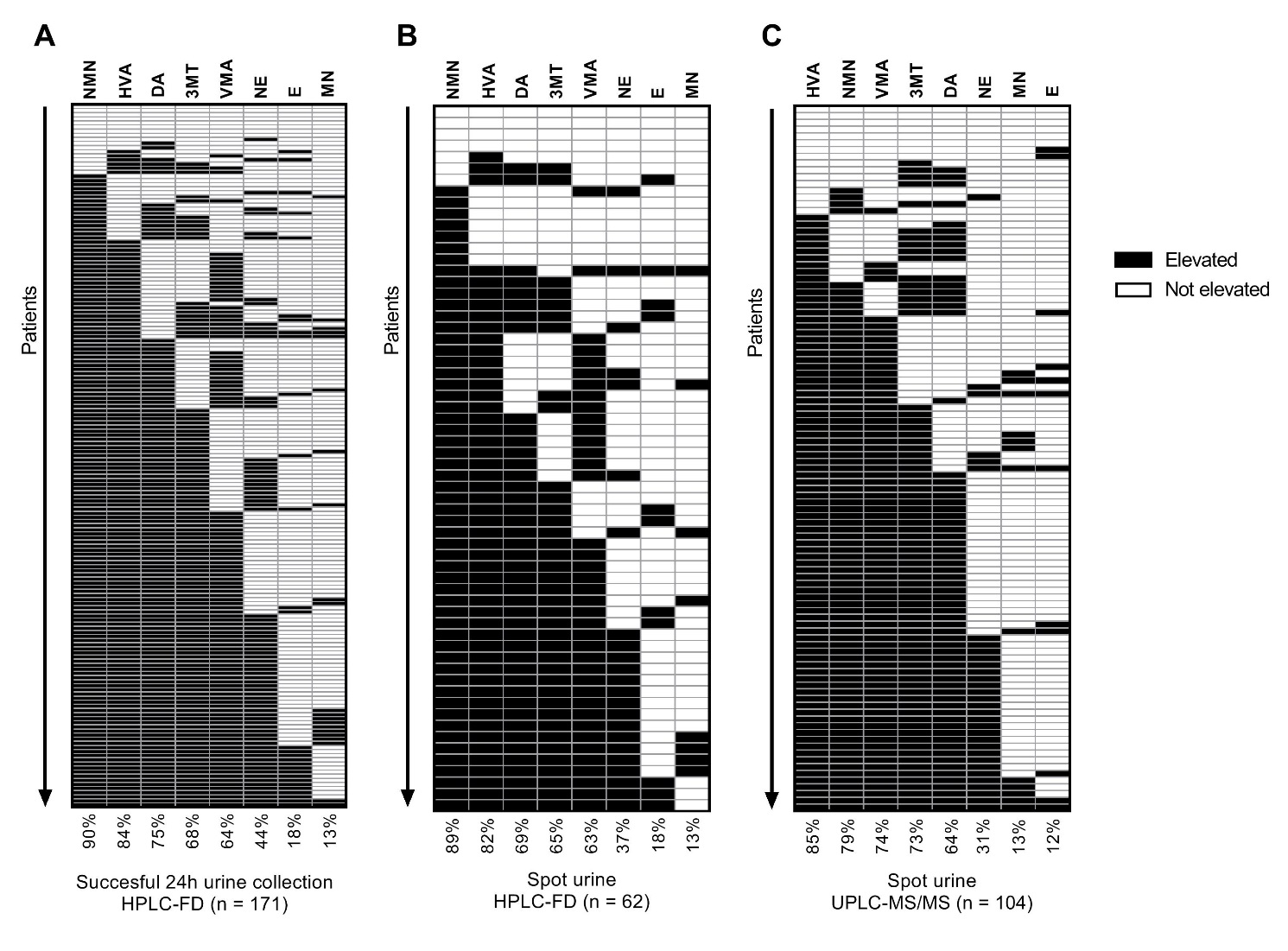
Neuroblastoma and non-neuroblastoma (i.e., patients with other paediatric cancer types or no malignancy) spot urine samples (n = 39) received for catecholamine analysis were analysed by both HPLC-FD and UPLC-MS/MS. Concentration of urinary catecholamine metabolites were normalized to creatinine concentration. Every dot represents a spot urine and the line indicates the best fit according to linear regression performed on the whole group. A = homovanillic acid (HVA), B = vanillylmandelic acid (VMA), C = dopamine (DA), D = 3-methoxytyramine (3MT), E = norepinephrine (NE), F = normetanephrine (NMN), G = epinephrine (E), H = metanephrine (MN). HPLC-FD, High Performance Liquid Chromatography coupled with fluorescence detection; UPLC-MS/MS, ultra-performance liquid chromatography coupled with electrospray tandem mass spectrometry

**Supplementary Figure 3** Effect of the analysis method on diagnostic sensitivity for neuroblastoma

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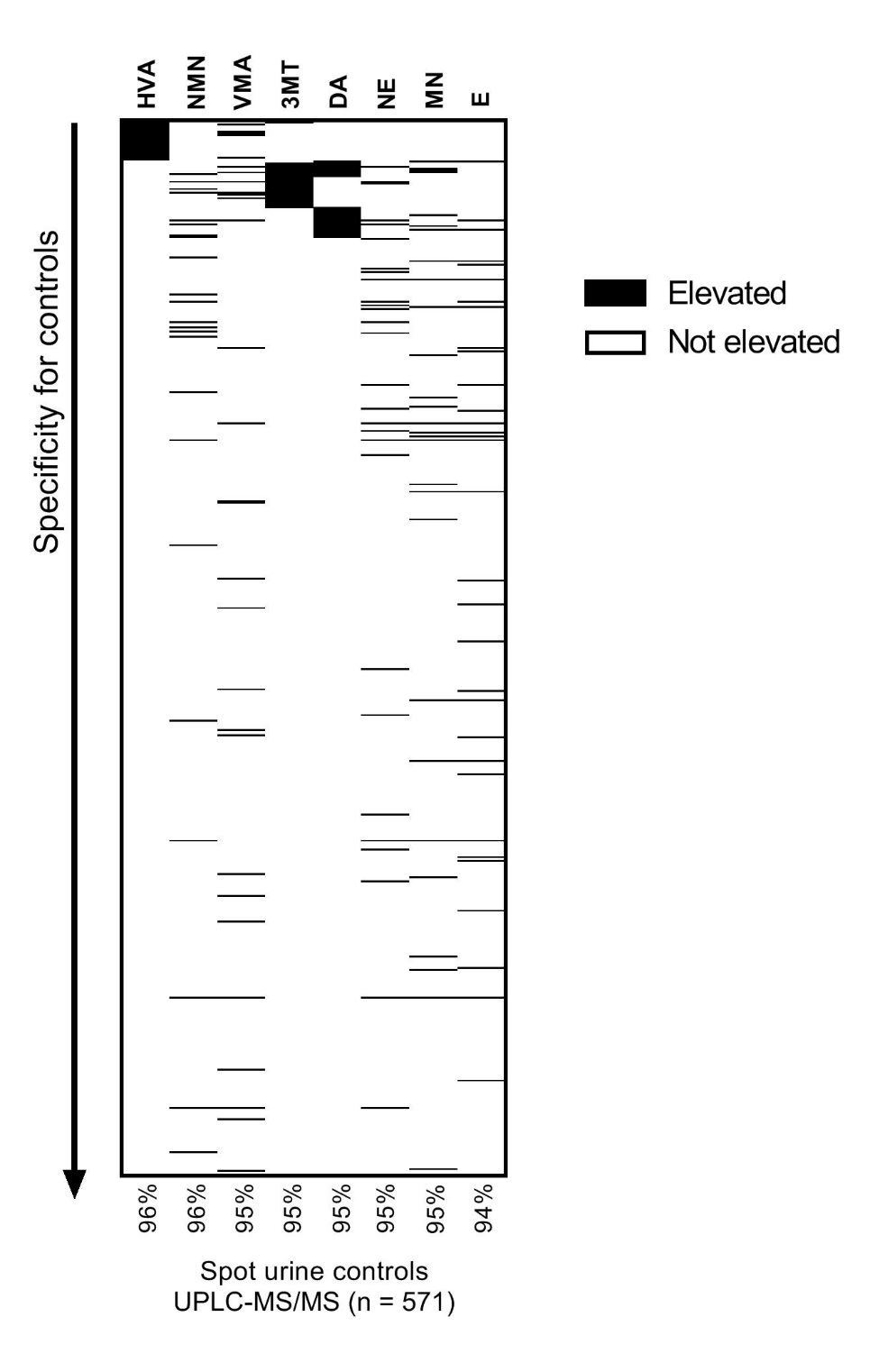
Urine was collected at diagnosis as spot urine in the Amsterdam UMC and analysed by HPLC-FD (n = 62, black) or by UPLC-MS/MS in the Princess Maxima Centre (n = 104, white). Diagnostic sensitivity was defined as the number of patients with elevation of this specific metabolite divided by total number of patients. P-values are given.

**Supplementary Figure 4** Diagnostic sensitivity of a panel of eight urinary catecholamine metabolites for the diagnosis of neuroblastoma



Every row indicates a different patient and every column a different metabolite. Every patient was tested for the whole panel. Whether or not the metabolite was elevated is indicated by the colour black and white, respectively. A = diagnostic urines analysed by HPLC-FD in successful 24-hour (24h) urine collection, B = diagnostic urines analysed by HPLC-FD in spot urine, C = diagnostic urines analysed by UPLC-MS/MS in spot urine. The diagnostic sensitivity of every marker in the different groups is indicated below the heat map.

**Figure S5:** Specificity of a panel of eight urinary catecholamine metabolites for our controls



Every row indicates a different control and every column a different metabolite. The whole panel of catecholamine metabolites was investigated in each control. Whether or not a metabolite was elevated is indicated by the colour black (elevated) and white (not elevated). The diagnostic specificity of every marker is indicated below.

**References**

1. Verly IR, van Kuilenburg AB, Abeling NG, et al. Catecholamines profiles at diagnosis: Increased diagnostic sensitivity and correlation with biological and clinical features in neuroblastoma patients. *Eur J Cancer.* 2017;72:235-243.

2. Barco S, Verly I, Corrias MV, et al. Plasma free metanephrines for diagnosis of neuroblastoma patients. *Clin Biochem.* 2019;66:57-62.