Quantification of urinary neurotrophins by ELISA assay in prostate cancer patients

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INTRODUCTION

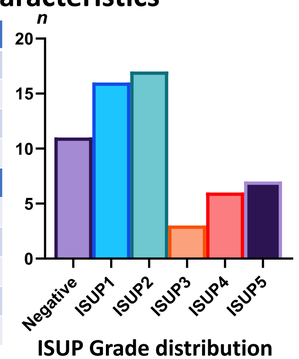
The autonomic nervous system regulates the development and progression of prostate cancer. Prostate cancer-derived neurotrophins promote nerve infiltration into the tumour microenvironment, whereby the local release of neurotransmitters stimulates cancer cell progression, growth, and invasion. Immunohistochemical studies have shown an association between prostatic neurotrophins (in particular BDNF, proNGF, NGF, GDNF)[1-3] and tumour aggressiveness, therefore we hypothesised that urinary neurotrophins may be candidate biomarkers for prostate cancer detection and prognosis.

METHOD – Specimen collection

- First-pass urine samples collected from men undergoing transrectal ultrasound guided prostate biopsy for investigation of suspected or established prostate cancer. Healthy men at low-risk of prostate cancer donated urine samples to be used as controls.
- Samples were collected and processed as per the Human Kidney and Urine Proteome Project protocol (ref). In brief, a urinalysis was immediately performed on collected samples, which where then on ice and transferred to the laboratory for processing. Samples were centrifuged at 1000g, 4degC for 10 min, aliquoted and stored at -80degC within 6 hours of collection. No protease inhibitors or anti-bacterial agents were added.

TABLE & FIGURE 1 – Patient characteristics

Demographics	
Age *mean (range)*	65 (28-79)
Biopsy *n (%)*	60 (80%)
Controls	15 (20%)
Total specimens	75
Biopsy characteristics	
Negative *n (%)*	11 (18%)
Prostatitis	5 (8.3%)
Positive	49 (82%)
Perineural invasion	17 (28.3%)
PSA *mean (std dev)*	9.8 (10.4)



METHOD – ELISA protocol

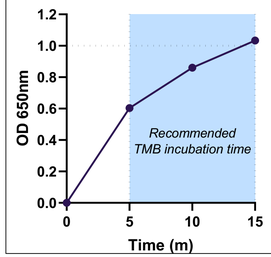
- Biosensis Rapid™ ELISA kits were used to assay NGF, BDNF, NT3, NT4/5, proNGF, proBDNF and GDNF. These kits area a sandwich ELISA that consist of a pre-coated capture antibody, a biotinylated detection antibody, and horseradish peroxidase-conjugated streptavidin. Addition of a substrate (TMB) yields a colour reaction directly proportional to the concentration of the specific neurotrophin in the sample or standard.
- Spike recovery, and linearity tests were performed to establish a minimum required sample dilution (MRD) (*Figure 3*). Samples were thawed on a 37degC heating block, and diluted to the MRD with kit-supplied diluent.
- The optimal TMB incubation time for our laboratory was determined by serial 650nm absorbance measurements of the blue TMB colour reaction, and the stop solution was added when OD values of the highest standard were above 1.0.
- Total protein *(Thermofisher BCA assay)* and creatinine (*Cayman Chemicals Urinary Creatinine colorimetric* assay) were measured to normalise neurotrophin concentration, in order to adjust for variability in dilution between samples.
- Difference in mean neurotrophin concentration between patient groups was assessed using a Student's t test with Welch's correction.

TABLE 2 – RESULTS *(measurements in pg/mL)*

NTF		Range		No cancer		Cancer		Significance	
	n	Min	Max	n pos	mean	n pos	mean	Mean diff.	p-value
NGF	75	<LOD	52.2	7/26	5.9	23/49	6.0	0.18 ± 3.0	0.95
BDNF	61	<LOD	25.8	13/22	4.5	25/39	4.2	-0.21 ± 1.5	0.89
NT3	60	<LOD	51.6	12/22	11.5	14/38	10.7	-0.78 ± 3.7	0.83
NT4/5	75	77.7	924.4	26/26	307.8	49/49	287.0	-20.9 ± 38.9	0.59
proNGF	38	<LOD	3.6	8/11	0.9	12/27	0.5	-0.42 ± 0.36	0.27
proBDNF	75	<LOD	253.2	16/26	36.1	32/49	36.5	0.34 ± 13.7	0.98
GDNF	36	<LOD	71.3	12/13	13.4	23/23	13.7	0.26 ± 4.0	0.95

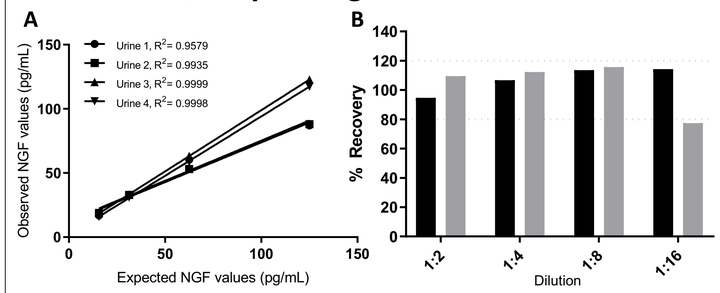
- Neurotrophins were detected in both cancer and healthy urine, but there was no significant difference in the mean concentration between groups in any of the tested neurotrophins.
- Normalising the neurotrophin concentration to urinary creatinine or total protein concentration did not result in a significant difference between cancer positive and negative groups.
- No correlation was observed between neurotrophin concentration and prostate cancer aggressiveness, as measured by ISUP Grade group.

FIGURE 2 – TMB optimisation with NGF ELISA kit



Longer incubation times were required for our laboratory conditions in five out of the seven kits tested. NGF (pictured left) achieved reached adequate absorbance within the recommended time frame.

FIGURE 3 – Quality testing with NGF ELISA kit



Example of ELISA performance testing conducted in-house.

A. Linearity: Four urine samples were spiked with exogenous NGF standard, and serially diluted. All four samples demonstrated excellent linearity as demonstrated by linear regression.
B. Spike recovery: two urine samples were serially diluted from 1:2 to 1:16, then spiked with exogenous NGF standard. Acceptable recovery (between 80 to 120%) was achieved with a minimum required dilution of 1:2.

CONCLUSIONS

- Urinary neurotrophins can be reliably detected in human urine. Minimal sample matrix interference yields a high degree of linearity and spike recovery, allowing accurate quantification at low dilutions.
- We have not evidenced any significant difference between prostate cancer patients, and those without cancer.
- Optimisation of TMB incubation times is advisable to achieve the recommended standard curve absorbance values.

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