ß-Trace protein for the detection of CSF leaks; comparison with ß2-transferrin

Boscato L[1], Manaf C[2], Bayoun R[1], Jones GRD[1].
Departments of Chemical Pathology[1] and Immunology [2], St Vincent's Hospital, Sydney

Introduction and Aim

- ß2- transferrin (B2T) and ß trace protein (BTP) are markers for the diagnosis of CSF leakage[1].
- B2T is not a valid test for CSF in fluids contaminated by bacterial sialidase[2], or high levels of serum indicated by high protein concentrations. Such samples account for 36% of specimens received in our laboratory.
- The aim of this study was to determine if BTP could be used to identify CSF in a wide range of clinical samples.

Methods Summary

Validation of the BTP assay: matrix effects, sialidase and haemolysis interference, precision, linearity and detection of CSF in serum.

2. Determination of expected values for fluids: serum, CSF, nasal fluids. Effect of renal failure on serum BTP level.
3. Comparison of B2T and BTP results for fluids received for B2T assessment from patients suspected of having a CSF leak.

BTP was measured using the NLatex BTP on the Dade Behring BN ProSpec nephelometer.

B2T was detected by isoelectric focussing followed by blotting and immunodetection.

Results Summary

1 • BPT recovery is acceptable indicating that the assay is not significantly affected by sample matrix, sialidase presence or haemolysis (Figure 1).
- The assay is linear across the assay range (0.2-13mg/L)
- With a known serum and CSF concentration of BPT, CSF presence can be detected in serum to at least a 1/64 dilution (Table 1).

2 • Levels of BTP in serum and nasal fluid are significantly lower than CSF in subjects with normal renal function (Figure 2).
- Patients with renal failure can have serum BTP levels within the CSF range (Figure 3).
- BTP >1.35 mg/L (highest serum value +3 x analytical SD) in a fluid indicates CSF presence in a patient without renal failure. This is approximately 3-12% CSF depending on the actual serum and CSF levels of BTP.

3 • There was 100% agreement of BTP interpretation with B2T-ve and 94% with B2T +ve results.
- 32/37 B2T results that could not be reported gave clear results with BTP.

Discussion and Conclusions

- Lower levels of CSF can be detected in fluid specimens using BTP compared to B2T.
- Use of BTP eliminates the potential for false positives resulting from sialidase presence.
- BTP can be used for a wider range of specimens than B2T including samples contaminated with sialidase or having protein levels up to at least 30g/L with the following precautions.

  a. A paired serum sample should be obtained for analysis of protein, creatinine and BTP particularly if the specimen suspected of containing CSF is serous.

  b. Care should also be taken with results interpretation for low volume specimens which may have concentrated.

1. Assay Validation

MATRIX AND INTERFERENCE

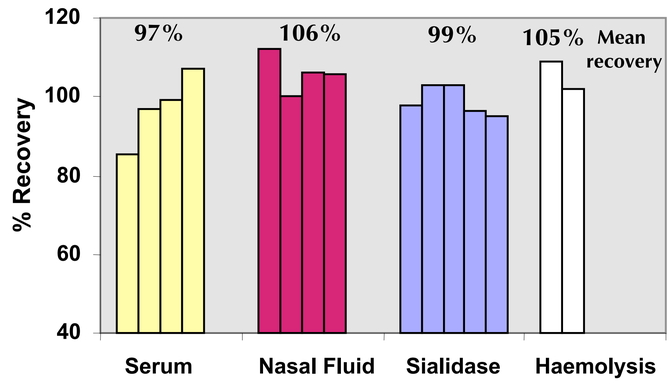


Figure 1. Recovery of BTP in the presence of serum and nasal fluids, sialidase activity and haemolysis.

Effect of sample matrix was investigated by adding a small volume of CSF to a number of serum and nasal fluid specimens and determining % recovery of the added BTP. Sialidase interference and heamolysis were assessed by determining recovery of BTP in CSF spiked with sialidase activity (5 samples) and red cell haemolysate (final Hb 996 and 5423 mg/dL) respectively.

LINEARITY

Table 1. CSF was serially diluted in serum and assay diluent to investigate both assay linearity and sensitivity of BTP detection. The highest dilution of CSF in serum to have a BTP significantly higher than the serum alone is shown(*)

CSF Dilution	% Serum	BTP (mg/L)			% recovery	
		Serum a	Serum b	Diluent	Serum	Diluent
Neat	0	18.2	23.7	23.7		
1/8	88	2.61	3.36	2.91	106	98
1/16	94	1.61	1.93	1.44	94	97
1/32	97	1.13	1.24	0.74	65	100
1/64	98	0.86 *	0.97	0.4	98	109
1/128	99		0.74 *	<0.2	95	
	100	0.6	0.57	0		

Mean recovery of all dilutions of BPT in diluent 103% and serum 98% (not all data shown).

PRECISION

Between run precision was determined using in-house and commercial controls. Low: mean 0.6, %CV 2.58, n=15; Medium: mean 1.53,%CV 7.9, n=19; High: mean 8.67, %CV 3.07, n=18.

2. Expected values

BTP LEVELS IN VARIOUS FLUIDS

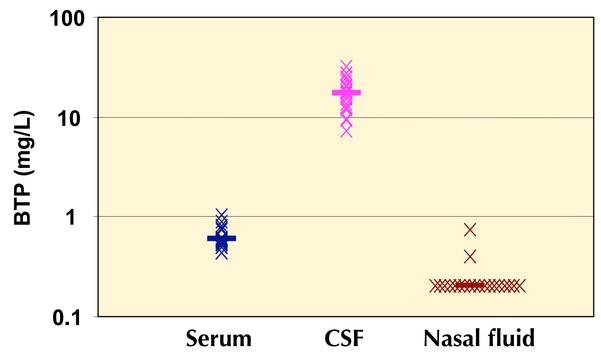


Figure 2. BTP levels obtained for serum, CSF and nasal fluid specimens.

Serum and CSF specimens were selected from samples received for routine analysis (serum -low creatinine, CS F- normal biochemistry and bacteria free). Nasal fluids were chosen from samples received for B2T analysis but found to be B2T free and of low protein content. BTP levels below in mg/L.

Serum **n= 25, range 0.45-1.05, mean 0.606**
CSF **n = 29, 69% >13.2 (assay upper limit), 22 samples quantified, range 7.2 – 31.9, mean 17.4**
Nasal fluid **n= 20, 18/20 <0.2 (assay lower limit) range <0.2-0.7**

BTP LEVELS IN RENAL FAILURE

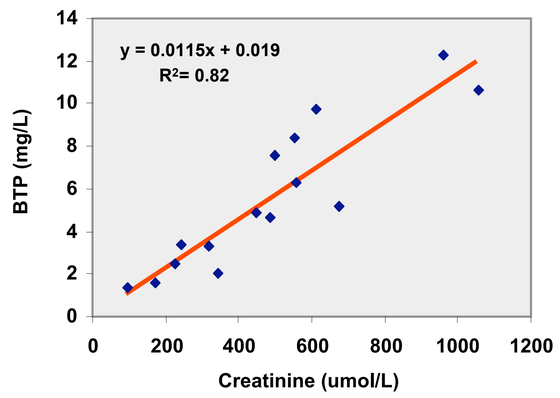


Figure 3. BTP levels obtained for serum specimens with elevated creatinine concentrations.

To investigate the effect of renal failure on serum BPT levels Serum specimens from patients with renal failure (elevated creatinine) were assayed.

3. Comparison of B2T and BTP results

95 samples that had been received for B2T analysis were assayed for BTP. B2T and BTP results are given below.

B2T REPORTABLE SAMPLES

58 samples were able to be reported for B2T. The B2T and the BTP results are shown below

	BTP -	BTP +
B2T-	23	-
B2T+	2	33

Comment: two B2T+ve samples gave negative BTP results (Protein 3-10 mg/L) - Possible missed sialidase interference in B2T assay.

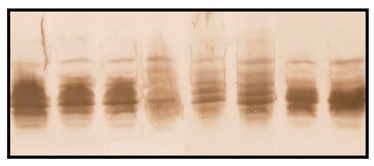
SIALIDASE INTERFERENCE

10 low protein samples (<15g/L) were reported as suspected sialidase interference (all had B2T band-Refer Fig 4 b) all gave BTP < 0.2mg/L (-ve).

HIGH PROTEIN

27 samples with protein >15g/L could not be reported for B2T due to high protein. 22/27 were tested for B2T and all had a B2T band (refer Fig 4. a, d).

BTP < 1 (-ve) 13 samples
BTP 1-3 5 (protein>30g/L)
BTP >7 (+ve) 9 samples



	Dilution	Protein (g/L)	BTP (mg/L)
A	1/50	55	0.48
B	½,1/4	low	0.89
C	1/2	CSF	>13.2
D	1/60, 1/120	41	0.83
E	½,1/4	low	>13.2

Figure 4. Immunoblot showing transferrin isoforms for samples a-e. Sample dilution, protein and BTP content are given

All samples have a B2T band (uppermost band) and could be interpreted as containing CSF. BTP was only elevated in two samples.

Acknowledgment. This study was funded by a SydPath Research grant

References
1. **Arrer E et al. Clin Chem 2002;48: 939-941.**
2. **Boscato L et al. Clin Biochem Revs 1994:15 Sept 135.**