Heparin-binding protein as a marker of ventriculostomy related infection and central nervous system inflammation in neuro-intensive care

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Objective: Diagnosis of ventriculostomy related infections (VRI) in the neuro-intensive care unit remains challenging and current biomarkers lack adequate precision. The aim of this study was to explore the potential of heparin-binding protein (HBP) in cerebrospinal fluid (CSF) as a diagnostic biomarker of VRI.

Methods: All patients treated with an external ventricular drain (EVD) between January 2009 and March 2010 at Skåne university hospital in Lund, Sweden, were consecutively included. CSF samples obtained during routine care were analyzed for HBP. VRI was defined as a positive bacterial microbiology test result on a CSF sample with an erythrocyte-corrected leukocyte count of $> 50 \times 10^6$/l. HBP levels at VRI diagnosis was compared to peak HBP levels in non-VRI controls.

Results: In total, 394 CSF samples from 103 patients were analyzed for HBP. Seven patients (6.8%) fulfilled VRI criteria. Levels of HBP were significantly higher in VRI subjects (31.7 ng/mL [IQR 26.9–40.7 ng/mL]) compared to non-VRI controls (7.7 ng/mL [IQR 4.1–24.5 ng/mL]) ($p = 0.024$). The AUC of the receiver operating characteristic (ROC) curve was 0.76 (95% confidence interval [CI], 0.62–0.90). Among non-VRI patients, HBP was highest in patients with acute bacterial meningitis. Patients with subarachnoid hemorrhage displayed higher HBP levels than those with traumatic brain injury or shunt dysfunction.

Conclusions: HBP levels were higher in VRI subjects and varied between patients and different diagnoses. To validate the clinical usefulness and added value of HBP as a biomarker for VRI, the results need to be confirmed in larger studies with head-to-head comparisons to current biomarkers.

1. Introduction

The external ventricular drain (EVD) or ventriculostomy catheter, remains a central device in neuro-intensive care. It allows monitoring of intracranial pressure, cerebrospinal fluid (CSF) sampling as well as drainage to lower intracranial pressure [1]. The use of EVDs carries a risk of healthcare-associated central nervous system (CNS) infections, such as bacterial meningitis or ventriculitis [2]. Infections related to an EVD are called ventriculostomy related infections (VRI) or EVD-related infections (EVDRI). VRI can lead to further brain damage, worse clinical outcome or prolonged hospital stay [3] and should be diagnosed and treated early. The current diagnostic gold standard is bacterial culture of the CSF. This assay is dependent on bacterial growth rate and analysis often takes several days. Hence, empirical treatment is often initiated based on symptoms and analysis of CSF biomarkers such as leukocyte count and differential, lactate, protein, and CSF/plasma glucose ratio. However, symptoms and biomarker changes associated with a CNS infection can be similar to those caused by the underlying neurosurgical or neurological conditions [4,5]. This leads to both over-use of broad-spectrum antibiotics and delay of diagnosis [6]. There are also cases where symptoms and biomarker changes are highly indicative of infection without alternative explanations and CSF culture still yields a negative result. This causes concern that the sensitivity of CSF culture may be limited under some conditions, such as ongoing antimicrobial treatment for other infections.

Other CSF biomarkers have been investigated to find a more robust protocol for VRI diagnosis. A cell-index, where ratios of leukocytes to erythrocytes in CSF and blood are compared has been shown to increase

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the diagnostic performance of the CSF leukocyte count [7–10]. However, recent research has shown that leukocyte counts differ significantly in repeated samples due to sedimentation in the CSF questioning the use of this biomarker altogether [5]. Lactate has been proposed as a more specific CSF biomarker for nosocomial meningitis in small studies with varying definitions of infection [11–13]. However, the largest study of lactate in VRI, specifically, questioned the reliability of the biomarker [14]. There are also studies of other biomarkers such as interleukins, tumor necrosis factor-α (TNF-α) and Procalcitonin in CSF and plasma with promising results [15–21]. However, the differences in VRI definitions between studies decreases comparability [22,23] and so far, none of these novel biomarkers have been put into widespread clinical use.

Heparin-binding protein (HBP) is a protein stored in azurophilic granules and secretory vesicles of neutrophils and is released upon activation of neutrophils [24]. HBP has been studied in several settings, primarily in sepsis where it has been shown to correlate with organ dysfunction and mortality [25,26]. It has also been shown that CSF HBP is elevated in community acquired acute bacterial meningitis (ABM) but not in viral CNS infections or CNS infection with Borrelia burgdorferi [27–29]. These findings, in addition to the fact that HBP can be measured simply and accurately, even with point-of-care methods [30], have raised the question whether CSF HBP could be useful in neuro-intensive care to provide improved diagnostic accuracy for VRI.

Kong and co-workers have recently shown that HBP can serve as a biomarker for nosocomial meningitis and ventriculitis, including VRI, with superior diagnostic accuracy compared to CSF lactate and CSF procalcitin [31]. The primary aim of this study was to investigate how HBP in CSF is related to VRI in a cohort of mixed neuro-ICU patients. Secondary aims were to investigate if HBP levels differ between different conditions in neuro-intensive care and how HBP concentrations correlate to CSF leukocyte count since this affects its added value as a biomarker.

2. Materials and methods

2.1. Study design and setting

This was a retrospectively designed observational study on a prospectively collected dataset on HBP in CSF of neuro-ICU patients, investigating this biomarker in relation to CNS infection and inflammation. Between January 2009 and March 2010, all patients treated with an EVD that had CSF sampled via the EVD in the neuro-ICU at Skåne university hospital in Lund, Sweden, were included consecutively. Lund is the regional center for neuro-ICU care for southern Sweden and the unit has a catchment area of approximately two million inhabitants and is supported daily by infectious disease specialists. Data regarding age, gender, cause of admission, duration of care, number of CSF-samples collected and administered antimicrobial treatment were collected from electronic patient records.

2.2. EVD-catheters and CSF sampling

EVD insertion was performed in the operating room under sterile conditions. Polyurethane EVDs (Liquor Drainage Catheter Set (Smiths Medical Deutschland GmbH, Grasbrunn, Germany)) without antibacterial coating were used. Catheters were inserted by a frontal burr hole and tunneled subcutaneously approximately five cm from the incision site. The EVD was connected to an external draining system with a pressure monitoring device (Hanni-Set (Smiths Medical Deutschland GmbH, Grasbrunn, Germany)). Periprocedural antibiotics were not routinely administered. The catheters were not routinely exchanged. A closed ventriculostomy system was used and CSF samples were collected routinely twice per week and when there was a clinical suspicion of CNS infection such as fever, increase of inflammatory biomarkers or worsening of neurological status without other clinical explanations.

2.3. CSF parameters

For each included CSF sample, results of CSF cell count separated in erythrocytes, polymorphonuclear and monomorphonuclear leukocytes, CSF glucose and CSF protein were collected from electronic patient records. When available, results of plasma CRP and blood total leukocyte count from the same day as CSF sampling, were also collected. These analyses were performed at the laboratory of Clinical Chemistry at Skåne University Hospital in Lund by standard clinical procedure. The samples were frozen and stored for subsequent HBP analysis. When available, results of CSF culture and polymerase chain reaction (PCR) for bacteria were collected. These analyses were performed by standard clinical procedure at the laboratory of Clinical Microbiology at Skåne University Hospital. HBP concentration was analyzed in duplicates by an enzyme-linked immunosorbent assay (ELISA) as previously fully described [32]. The person analyzing the samples was blinded regarding the identity and clinical characteristics of the subject.

2.4. VRI definition

In this study, VRI was defined as a positive culture or bacterial polymerase chain reaction (PCR) on CSF in combination with CSF-procalcitonin, defined as an erythrocyte-corrected leukocyte count (leukocyte count – erythrocyte count/1000) of > 50 × 10^6/L. This is in line with the recommendations from Lozier et al. to include both a microbiological criterion and evidence of CNS inflammation when defining VRI [23]. Subjects with positive CSF culture or PCR but without pleocytosis were defined as contaminations and included in the non-VRI-group. Subjects with a confirmed infection (ABM or ventriculoperitoneal shunt infection [SI]) as the indication for EVD-placement were not classified as either VRI or non-VRI and excluded from the group comparisons.

2.5. Statistical analysis

Subjects with positive microbiology results defined as contaminations were included in the non-VRI group. The HBP value of the sample with the first positive microbiological test result classified as VRI was used for subjects in the VRI group. The peak HBP value in samples collected without ongoing VRI treatment was used for the non-VRI group. Samples collected during VRI treatment for control of treatment response or due to clinically uncertain diagnosis were excluded as there is a risk of false negative cultures in these samples. Also, samples collected during VRI treatment cannot be considered diagnostic since treatment has already been initiated. For comparison of HBP levels in different diagnoses, diagnose groups with at least 3 subjects were included and the peak HBP value for each subject was used. To reduce the impact of VRI on HBP-levels in this analysis, VRI subjects were excluded. For the analysis of the correlation between CSF HBP and CSF leukocytes, all samples were included in a Spearman’s rank correlation model. Results are presented as medians (interquartile ranges [IQR]) or medians (range). The Mann–Whitney U-test was used for all group-wise comparisons. P-values < 0.05 were considered statistically significant. The optimal cut-off value for CSF HBP was calculated analyzing the receiver operating characteristic (ROC) curve. Statistical analysis and graphic visualization were performed using R version 4.1.0 and R studio version 1.4.1717.

2.6. Ethical considerations

HBP levels were not available to the treating physician and hence, the study did not affect the medical management.
3. Results

3.1. Study population characteristics

In total, 103 subjects were included in the study. Seven patients were categorized as VRI and 83 as non-VRI. The 13 remaining subjects had ABM or SI were not categorized as neither VRI nor non-VRI. A flow chart illustrating the group categorization is presented in Fig. 1. Basic epidemiological data, admission diagnosis and CSF and blood parameters of the VRI and non-VRI group are presented in Table 1. HBP was analyzed on 405 CSF samples collected during the study. Twelve CSF samples were not analyzed for HBP for various reasons, leaving 393 samples for analysis. A total of 109 samples were collected when no antibiotic treatment was given and 284 were collected during antibiotic treatment including 144 that were collected during antibiotic treatment for VRI.

3.2. Microbiology

Bacterial culture or PCR was performed on 363 of 393 CSF samples. Microbiological results and their interpretation are displayed in Table 2. Culture was positive in 34 samples drawn from 26 subjects. PCR for 16 S rDNA or specific PCR was performed on 47 samples and was positive in 10 cases. Of the 10 PCR positive samples, 2 (1 Acinetobacter species and 1 Neisseria meningitidis) were not connected to a positive CSF culture, but in one of these cases the same microorganism (Acinetobacter species) was cultured in a previous sample. In total, 36 samples (9.9% of the investigated samples) from 27 subjects (26.2% of total subjects) tested positive for the presence of bacteria. Out of these, 14 samples were from 7 subjects that were classified as VRI, and 13 samples were from 9 subjects where the microbiological findings were interpreted as contaminations and subjects classified as no VRI. The remaining 9 positive samples were from subjects with either ABM or SI. Two subjects had divergent microbiological findings in different samples (one with alfa-streptococcus species and Enterobacter cloacae and one with Streptococcus oralis and Candida albicans). The 7 subjects with VRI had findings of coagulase negative staphylococci (CoNS) in 5 cases, Escherichia coli in 1 case and Streptococcus oralis followed by Candida albicans in the last case. The subject with two different pathogens at different times fulfilled the criteria for VRI on both occasions. In 9 subjects, positive microbiological results were classified as contaminations. These subjects had findings of CoNS in 6 cases, Micrococcus species in 2 cases and Streptococcus mitis in 1 case.

3.3. HBP in subjects with VRI

HBP at VRI diagnosis was compared to peak HBP in samples from non-VRI subjects without ongoing VRI-treatment (Fig. 2). HBP was
3.4. HBP in relation to diagnosis

Peak CSF HBP levels in different diagnoses were compared. VRI subjects were excluded. Results are displayed in Fig. 4. Peak HBP levels were significantly higher in the ABM group than in the SAH group (p = 0.026), which in turn were significantly higher than the SD and TBI groups (p = 0.0010 and p = 0.00086, respectively).

3.5. HBP correlation to CSF leukocytes

Using Spearman’s rank correlation on all CSF samples where both HBP and leukocytes had been analyzed (n = 385) Spearman’s rho was 0.65, 0.61 and 0.63 (p < 2.2 × 10^{-16}) for total-, polymorphonuclear- and monomorphonuclear leukocyte count respectively.

4. Discussion

This study is, to the best of our knowledge, the second study investigating the potential role of the neutrophil derived Heparin-Binding Protein (HBP) in CSF as a biomarker of nosocomial CNS infection and the first to specifically study it in ventriculostomy related infections (VRI). CSF HBP levels were statistically significantly higher in subjects with VRI, despite the low number of subjects in the VRI group, indicating a relevant effect size. The AUC of the ROC curve and the sensitivity and specificity calculated were limited compared to the results of Kong et al. [31] and it is still unclear if HBP would complement current diagnostic biomarkers. The results also show that HBP levels vary between patients and diagnoses and that HBP concentrations are higher in acute bacterial meningitis (ABM) compared to other diagnoses studied. HBP concentrations in patients with subarachnoid hemorrhage (SAH) were also shown to be significantly higher than in patients suffering from shunt dysfunction (SD) and traumatic brain injury (TBI). Finally, it was shown that CSF HBP only has a moderate correlation to leukocyte counts in CSF. We believe these findings indicate that HBP could potentially serve as a clinically relevant biomarker for VRI. This needs to be confirmed in future studies before clinical implementation is considered given the additional costs and increased complexity that introducing another biomarker would bring.

A recent study of Kong and co-workers has shown excellent diagnostic performance of CSF HBP in nosocomial meningitis and ventriculitis, indicating that HBP may be useful in this setting [31]. However, their results were more encouraging than ours regarding the diagnostic accuracy of CSF HBP with an AUC of the ROC-curve of 0.99 compared to the more modest AUC of 0.76 in our study. In the sub-group analysis of VRI in their study, the sensitivity and specificity for CSF HBP > 21 ng/mL was 100% and 96% respectively which is considerably higher than what we estimated based on our material. Differences in study design could explain the differences in results. Although their study is substantially larger, increasing the statistical power, the study population is primarily composed of patients treated for CNS-tumors which may reduce the applicability of the results to other patient groups. Another important difference is how VRI was defined. The definition used by Kong et al. allowed VRI to be diagnosed based on clinical symptoms and CSF-cytochemistry (leukocyte count > 10^6/l, protein > 500 mg/L and glucose < 2.5 mmol/l) even without microbiological evidence of infection, whereas we used a definition requiring both a positive culture or PCR and pleocytosis. We believe this is a strength in our study and an important contributing factor to the more modest diagnostic accuracy of HBP in our material. The definition of pleocytosis of > 50 × 10^6 leukocytes/l is arbitrary and could be discussed. However, according to our clinical experience we believe it is a reasonable level that balances the risk of including irrelevant microbiological findings to the risk of overestimating HBP's diagnostic accuracy due to its correlation to CSF leukocytes.

Using only a cytochemical criteria for VRI diagnosis will over-diagnose infection since sterile inflammation will be interpreted as...
infection. The approach of specifying samples with marked pleocytosis as infection will also likely overestimate the diagnostic accuracy of biomarkers that are related to leukocytes, such as HBP or various cytokines. It could be argued, however, that a strict requirement of a positive culture or PCR to diagnose VRI clinically risk leading to missed diagnoses due to limitations in the sensitivity of current microbiological methods.

Another approach would be to define VRI strictly by a positive microbiological result. This, however, risk misclassifying contamination or colonization as infection which could negatively impact the assessment of the biomarker’s diagnostic accuracy and lead to an under-estimation of the biomarker’s sensitivity. In particular when the microbiological spectrum is dominated by low virulent bacteria like CoNS, as these pathogens trigger lower inflammatory responses and, likely, lower levels of inflammatory substances. In a meta-analysis of VRI-studies it was shown that Staphylococcus epidermidis and other CoNS are the most common VRI-pathogens [33] which we believe increases the clinical relevance of our results. Some studies of VRI have described a microbiological spectrum containing more virulent pathogens such as Staphylococcus aureus and gram-negative bacteria [7,34]. It will likely be easier to demonstrate diagnostic accuracy of a VRI biomarker in such a setting.

CSF HBP showed a moderate correlation to CSF leukocytes which was expected. It was, however, unexpected that the correlation was not very different when separating leukocyte count into polymorphonuclear and monomorphonuclear leukocytes as one would expect that a neutrophil derived protein such as HBP would be more strongly correlated to polymorphonuclear leukocytes. The correlation between HBP and leukocyte count was weaker in our material than in the study of Kong et al. [31] (Spearman’s rho of 0.65 compared to 0.81) but still stronger than the correlations reported in previous studies on HBP in ABM that have reported Spearman’s rho of 0.3–0.4 [27,28]. It is unclear what explains the differences between these materials. The fact that the correlation is not total in any of the studies could support the theory that HBP concentrations reflect the number of activated neutrophils and thus increase the likelihood of an added clinical value of HBP in VRI diagnostics.

This study has several limitations. The biggest being the limited number of included patients and the low number of subjects with VRI. However, we believe the fact that a statistically significant difference in CSF HBP between VRI and non-VRI subjects could be demonstrated in a material with such a low number of VRI subjects is encouraging and increases the relevance of performing larger studies on HBP in this setting. Our study cohort was relatively heterogenous regarding cause of admission. This could be a limitation since HBP’s characteristics may differ between different conditions and our small sample-size does not allow for meaningful sub-group analyses between different conditions. Another limitation is the fact that no head-to-head comparison to other biomarkers were performed which makes it harder to interpret the results and demonstrate added clinical value of implementing HBP clinically. Due to the inclusion of CSF leukocytes in our definition of VRI, no comparison to this parameter would be scientifically sound. At the time of this study, analysis of CSF lactate was not performed in our center and CSF glucose and protein alone are too unspecific to be used as independent diagnostic biomarkers.

5. Conclusion

In this study, HBP was shown to be significantly higher in VRI subjects. However, this study showed a more limited diagnostic accuracy than in the previous study of HBP as a biomarker of nosocomial ventriculitis and meningitis. Also, the low number of VRI patients included limits the significance of the results and the clinical value of HBP in this setting is still unclear. That significant results could be observed even in such a small cohort motivates further studies on HBP as a biomarker of infection in the neuro-ICU. Such studies should preferably be larger, feature a strictly defined protocol for inclusion, include head-to-head comparisons to proposed and established biomarkers and use a pre-defined definition of VRI based on microbiological findings in combination with cytochemical evidence of CSF inflammation.

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Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Regional Ethical Review Board in Lund (LU-2017/06).

CRediT authorship contribution statement

Johan Widen: Writing – original draft, Data curation, Formal analysis, Investigation, Visualization. David Cederberg: Conceptualization, Resources, Writing – review & editing. Adam Linder: Conceptualization, Resources, Writing – review & editing, Supervision, Investigation. Gabriel Westman: Writing – review & editing, Formal analysis, Visualization, Supervision.

Declaration of Competing Interest

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as hono-

raria; educational grants; participation in speakers’ bureaus; member-

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References

