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Haemolysis index: validation for haemolysis detection during extracorporeal membrane oxygenation

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Editor—Extracorporeal membrane oxygenation (ECMO)-induced haemolysis is provoked by blood trauma, release of gaseous microbubbles from degassing, or cavitation of red cells exposed to negative pressure (in the pump head, for instance).[1,2] When plasma-free haemoglobin (fHb)-scavenging mechanisms are overwhelmed, fHb may cause damage to the kidneys and other organs.[2–5] Therefore, prompt identification of haemolysis is of utmost importance as changes in therapy could be implemented. Measurement of fHb via the conventional spectrophotometry lacks automation, is cumbersome, and is, at best, performed only once a day during working hours. This predisposes to delayed detection of life-threatening haemolysis episodes.[6] Newer clinical chemistry analysers display a haemolysis index (HI; a dimensionless value) to assess the reliability of measurements potentially flawed by haemolysis (e.g. K^+).

The aim of this prospective study was to assess whether the HI, an *in vitro* tool proposed to detect haemolysis related to sample collection, transport, and processing, could be used to assess *in vivo* haemolysis in patients on ECMO. We also assessed (1) inter-measurement variability of HI and fHb and (2) impact on HI measurements of icterus and lipaemia (possibly related to propofol infusion) that may interfere with spectrophotometric measurements.[7] The ethics committee of the French Society of Anaesthesia and Intensive Care approved the study protocol (00010254-2016-038). The purely observational nature of this study and the lack of clinical data collected enabled patient consent to be waived.

In both derivation (March 2015–December 2016) and validation (January 2017–September 2018) cohorts, a daily blood sample from consecutive adult patients on ECMO at our institution was analysed for electrolyte analysis, and lithium heparin tubes were centrifuged at 2200 *g* for 10 min before analysing the supernatant. HI was measured via a Roche®-Cobas 6000 analyser (light absorbance tested at 570 and 600 nm). fHb measurements relied on spectrophotometry (Unicam UV3 UV/Vis Spectrophotometer; PerkinElmer, Hopkinton, Massachusetts, United States); after dilution (1:11) of a plasma sample, the absorption spectrum was measured at different wavelengths before and after reducing oxyhaemoglobin with sodium hydrosulphite.[8] The relationship between fHb and HI was assessed (Lin's concordance correlation coefficient [CCC]). In the *derivation cohort*, using receiver operating characteristics (ROC) curve analysis, we evaluated the ability of HI to detect fHb exceeding a critical threshold of 100 mg dl^{-1}.[3,6] In the *validation cohort*, we tested the cut-off of HI we determined in the derivation cohort. Beforehand, the bootstrap technique created a large set of 1000 samples in each cohort.

To assess the inter-measurement variability, the coefficient of variation of HI and fHb was determined in 30 repeated measurements on four home-made fHb solutions (25, 50, 100, and 200 mg dl^{-1}). Those solutions were prepared and frozen for a few weeks at −20°C after we ensured they were stable for more than 12 months. They were then thawed in a random order, and measurement of fHb and HI started simultaneously.

The Roche®Cobas 6000 analyser provides spectrophotometric measurements of icterus and lipaemic indices, surrogates for plasma total bilirubin and triglycerides, respectively.[7] An *in vitro* experimental exploration was performed in five patients. First, we evaluated the impact on HI of a gradual

increase of icterus index via manual addition of bilirubin. Second, we tested the impact of an increase in triglyceride concentration. Last, we tested the effect of a gradual increase in propofol concentration on the HI. Owing to the limited size of these preliminary experiments, no statistical comparisons were made. In our clinical cohort, a logistic regression assessed the performance of a model including HI, icterus, and lipaemic indices.

In the derivation cohort, 560 samples from 67 patients were analysed: median fHb was 3 mg dl^{-1} [interquartile range (IQR)=0; 20], ranging from 0 to 1992 mg dl^{-1} with fHb ≥100 mg dl^{-1} in 58 (10%) samples. Median HI was 5 [IQR=2; 12], ranging from 0 to 1655. There was a strong relationship between HI and fHb: CCC=0.93 [95% confidence interval (95% CI), 0.92–0.94]. For the detection of fHb >100 mg dl^{-1} (n=58 [10%]), the performance of HI was excellent: area under the ROC curve (AUC_{ROC}) of 0.98 (95% CI, 0.85–1.0), sensitivity=86% (95% CI, 57–100) and specificity=97% (95% CI, 91–100) for HI >20.

In the validation cohort, 700 samples from 95 patients were analysed. Median fHb was 4 mg dl^{-1} [IQR=0; 14], fHb ranged from 0 to 1526 mg dl^{-1}, with fHb ≥100 mg dl^{-1} in 50 (7.1 %) samples. Median HI was 4 [IQR=2; 8], ranging from 0 to 1011. There was also a strong relationship between HI and fHb (CCC=0.95; 95% CI, 0.95–0.96). For the detection of fHb ≥100 mg dl^{-1} (n=50 [7%]), HI ≥20 had good diagnostic performance: AUC_{ROC}=0.99 (95% CI, 0.95–1), negative predictive value 0.97 (95% CI, 0.93–1), positive predictive value 0.86 (95% CI, 0.50–1), rate of correct classification of 96% (95% CI, 92–99%).

The HI tended to have lower *inter-measurement variability* than fHb: for 25, 50, 100, and 200 mg dl^{-1} fHb solutions, the coefficient of variation was 9.4%, 9.0%, 6.6%, and 3.9% for fHb and 5.1%, 4.8%, 3.1%, and 0.4% for HI, respectively. *In vitro*, for a given degree of haemolysis (HI of 30), the gradual increase in icterus index (to 5, 10, 20, and 40) lowered the HI (−17%, −37%, −74%, and −100%, respectively). In contrast, in plasma with a baseline HI of 30, the gradual increase in lipaemic index (to 70, 125, 225, and 500) increased the HI (+11%, +14%, +25%, +56%, and +100%, respectively). The under-/overestimation of haemolysis by HI during severe icterus/hypertriglyceridaemia, respectively, was found with various degrees of haemolysis (baseline HI of 30, 60, 120, 250, or 500), but its magnitude tended to fall as haemolysis increased. Increasing propofol concentration (3, 6, and 10 μg ml^{-1}) had an irrelevant impact on the HI. In our derivation and validation cohorts, median icterus index and lipaemic indices were 2 [IQR=1–5] and 24 [IQR=15–42], respectively. A model including HI, icterus, and lipaemic indices did not outperform HI alone (AUC of 0.99 *vs* 0.99).

In our study, HI was a reliable marker of haemolysis during ECMO with low inter-measurement variability. A preliminary study (100 samples from in-house patients, not containing high degrees of haemolysis) found an excellent correlation (r=0.98) between HI and fHb.[9] This was confirmed by a recent retrospective study in patients on ECMO.[10] Our prospective study goes further than these studies because we included more samples from more patients (1260 samples from 162 patients). We also assessed the performance of HI for the detection of fHb below or above a critical threshold of 100 mg dl^{-1}.[3,6] We used a rigorous design with derivation and validation cohorts, assessed the inter-measurement variability of HI, and assessed the impact of icterus, hypertriglyceridemia, and propofol on the diagnostic performance of HI.

Limitations of the study include: (1) the study was monocentric; (2) caution should be exercised before extrapolating the good performance we report for HI to other chemistry analysers or to haemolysis not related to ECMO. However, the assessment of the degree of haemolysis seems to be similar from one analyser to another and observing a lower performance of the HI with other haemolysis triggers seems unlikely[7,11,12]; and (3) we did not collect clinical data because this preliminary study was not designed to assess the relationship between high HI and patient outcomes. Our encouraging results pave the way for such future studies.

In summary, as a fully automated, repeatable, and affordable gauge for ECMO-induced haemolysis, HI showed good performance and may replace non-routine measurements of fHb. Caution should be exercised in case of severe icterus and hypertriglyceridemia, but these conditions are also automatically quantified and displayed.

Declarations of interest

The authors declare that they have no conflicts of interest.

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Sugammadex or neostigmine: should potential anaphylaxis be the overriding factor in the choice of a reversal drug? Comment on *Br J Anaesth* 2020; 124: 154–63

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Editor—We read with interest the paper by Orihara and colleagues,[1] in which 49 532 patients from four tertiary hospitals in Japan were investigated to compare the incidence of anaphylaxis between sugammadex and neostigmine. Sugammadex is widely used in Japan: 18 cases of anaphylaxis were reported, of which six were attributable to sugammadex (0.02%; 95% confidence interval [CI], 0.007–0.044%) and none to neostigmine. The authors suggest that neostigmine is much less likely to cause anaphylaxis and that the choice of a reversal drug in Japanese practice should be reconsidered.[1]

Anaphylaxis related to general anaesthesia is a life-threatening complication and should be treated rapidly by well recognised management. The largest study of life-threatening perioperative anaphylaxis reported from the Royal College of Anaesthetists, 6th National Audit Project (NAP6), found an overall incidence of perioperative anaphylaxis (Grades 3–5) of 1:11 752 (95% CI, 10 422–13 303) and for sugammadex of 0.0016% (just one patient).[2] This is 10-fold less than that in the study by Orihara and colleagues,[1] although recently it has been argued that the two findings may not be dissimilar.[3] It is certainly difficult to ascertain the true incidence of any rare adverse event. The reported incidence of anaphylaxis to sugammadex in the literature is relatively low at 0.0016–0.039%.[1–5] Orihara and colleagues[1] stated that racial differences may be the cause of the lower incidence of sugammadex-induced anaphylaxis found in the NAP6 study. There are no data to support this statement. Furthermore, the exact mechanism of anaphylaxis from sugammadex alone or the sugammadex–rocuronium complex is still not well understood.[5] Previous sensitisation to sugammadex or similar compounds in the environment may also be a factor.[3]

The potential risk of anaphylaxis from any drug should be lowered by all possible means. Administration of a drug should be based on careful assessment of the indication and sugammadex is no exception. The only way to justify sugammadex administration and in particular its dose is to objectively monitor the level of neuromuscular block. There is some evidence from work in conscious volunteers that the risk of an allergic response to sugammadex increases with increasing dose.[6] However, no information is presented in the study by Orihara and colleagues[1] as to the doses of sugammadex used and whether neuromuscular monitoring had been undertaken in the patients. It is known that in clinical anaesthesia the application of neuromuscular monitoring is low globally and Japan is no exception.[7]

The incidence of anaphylaxis to neostigmine is unknown. According to the data sheet, neostigmine-induced anaphylaxis is rare to exceptional (<1/10 000). We performed a literature search in Medline (search criteria: 'neostigmine'/exp OR neostigmine AND 'anaphylaxis'/exp OR anaphylaxis, search date January 8, 2020) and identified 154 publications that met the search criteria. After screening the titles and abstracts, only six publications describing eight cases of anaphylaxis to neostigmine could be analysed.[8–13] In only five cases was neostigmine anaphylaxis confirmed by either measurement of plasma tryptase, skin testing, or both.[8–11] In the remaining three cases the suggested anaphylaxis to neostigmine was based on clinical signs only without further confirmation.[12,13] Hence, anaphylaxis from neostigmine is very rare, but it is not zero. However, from the existing data, it is impossible to even speculate on the incidence of anaphylaxis to this anticholinesterase. In cases of anaphylaxis after neostigmine administration, the drug should certainly be in the differential diagnosis as a potential trigger.

A recent Cochrane review showed that in comparison with neostigmine, sugammadex was able to reverse a rocuronium-induced neuromuscular block more rapidly regardless of the depth of the block.[4] Moreover, sugammadex reduced the signs of postoperative residual paralysis. Neostigmine is well recognised to take 10 min to reach its peak effect, and is unlikely to produce complete recovery from neuromuscular block.[14] Residual neuromuscular block after neostigmine is a contributing factor to the development of critical respiratory events in the PACU.[15]