Association between plasma-free hemoglobin levels, lactate dehydrogenase and hemolysis-index in patients with and without mechanical circuit support - a retrospective data analysis

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Research Article

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Abstract

Purpose

Hemolysis is a frequent problem in patients with mechanical circulatory support (MCS) that can indicate device malfunctions or pump thrombosis. In this retrospective cohort study, we assessed whether hemolysis index (HI) can replace plasma free hemoglobin (fHb) and lactate dehydrogenase (LDH) to guide anticoagulation and performed a cost analysis to combine medical with economical efficacy.

Methods

We correlated simultaneously obtained HI, fHb, LDH measures of venous blood samples in a cohort of 96 ICU patients. 429 datasets (40.7%) were collected from patients with and 556 datasets (52.8%) without extracorporeal life support (ECLS). 68 (6.5%) from a cohort of 51 outpatients on LVAD support. A cost analysis of all three hemolysis monitoring options was performed.

Results

Correlation of HI and fHb values was highly significant (r = 0.968, p < 0.01) overall and equally significant (r = 0.949, p < 0.01) in samples with clinically significant hemolysis (HI ≥ 20). Correlation of fHb and LDH was low (r = 0.192, p < 0.01) in all patients. Estimated cost per ICU patient on ECLS was 0 USD if HI, 4,347.25 (794.21-5350.46) USD if fHb, 383.29 (70.02-471.74) USD if serial measurements of LDH were used for hemolysis monitoring.

Conclusions

HI represents a cost-efficient, accurate and easily obtainable alternative to fHb measurements for clinicians treating patients with and without MCS, whereas LDH is significantly less specific. Further clinical studies on major cohorts of short and long-term MCS patients are needed to assess the optimal HI target with minimal thromboembolic as well as bleeding events.

Key Points

Question: We assessed whether hemolysis index (HI) can replace plasma free hemoglobin (fHb) and lactate dehydrogenase (LDH) to guide anticoagulation in patients with and without mechanically circulatory support (MCS).

Findings: In this retrospective cohort study correlation of HI and fHb values was highly significant (r=0.968), correlation of fHb and LDH was low (r=0.192). HI was highly cost effective compared to fHb and LDH if serial measurements were used for hemolysis monitoring.
Meaning: Unlike LDH, HI is a useful, cost-efficient and readily available alternative to fHb measurements and can be used for monitoring of anticoagulation in patients on MCS or with haemolytic conditions.

Introduction

Mechanical circulatory support (MCS) with extracorporeal membrane oxygenation (ECMO) or temporary extracorporeal right ventricular support (tRVAD) or/and extracorporal left ventricular support (LVAD) provide bridging options for patients suffering from acute heart failure, whereas implantable left ventricular assist devices (LVAD) provide support over prolonged periods of time.

However, pump- or outow graft thrombosis associated with hemolysis requiring thrombolysis or exchange of the circuit occur in right- or left ventricular assist devices and is detected in up to 23% patients on ECMO or tRVAD. Thromboembolism leading to cerebral stroke may cause long term disabilities and pump thrombosis and is a dreaded complication of extracorporeal MCS. Clinicians are often facing uncertainty while managing anticoagulation of these patients in situations with hypercoagulation such as sepsis or situations with gastrointestinal, pulmonary or cerebral bleeding. Thus, optimal anticoagulation with neither bleeding nor clot formation is crucial for long-term therapeutic success.

A valuable parameter for monitoring of thrombosis and hemolysis is plasma-free haemoglobin (fHb) concentration. It allows recognition of subclinical thrombosis and associated hemolysis and adjustment of anticoagulation before the occurrence of system failure or significant thromboembolic events. fHb released into plasma in a variety of diseases, including iatrogenic conditions – such as extracorporeal life support - can be measured as exceeding protective haptoglobin binding capacity. There is evidence linking increased fHb concentrations to pump thrombosis and acute kidney injury in adult and pediatric patients on ECMO as well as LVAD support.

Since use of short and long-term mechanical circulatory support (MCS) increases rapidly, treatment of patients with such devices become more frequent in less experienced centres which lack the ability to perform reliable fHb testing. Also experienced centres are under increasing economic pressure from hospital administrators and forced to optimize costs of expensive therapies and laboratory parameters. Due to cost cutting measures, fHb may be designated a routine laboratory parameter unavailable during off-peak hours.

Lactate dehydrogenase (LDH) is an intracellular enzyme involved in the conversion of lactate to pyruvate which is released into the serum when erythrocytes or other cells undergo lysis and thus levels of LDH have been associated with clinically significant hemolysis in patients suffering from hematological or infectious diseases. HI and fHb are unavailable in situations with very high serum bilirubin since hyperbilirubinemia interferes with photospectrometic HI and fHb measurement by altering light absorption by plasma. Unlike HI and fHb, LDH measurement is performed by measuring its catalytic activity via reduction of NAD to NADH, despite also involving absorption of light of NADH.
(photospectometry), it is very robust also in severe icterus and lipemia. Not only can LDH be used in situation when fHb is unavailable but previous investigations have shown significant increases in LVAD patients previous to pump thrombosis\textsuperscript{9,10}.

The hemolysis index (HI) is a quantitative or qualitative parameter measured automatically by laboratory instruments that perform routine blood analyses. It is not originally intended for clinical purposes, but is rather used to detect contamination of blood samples and interference of hemolysis on various analyses.\textsuperscript{14} HI is available on almost all machines performing routine clinical blood chemistry analyses and similarly to fHb is calculated based on the absorption of different wavelengths by serum or plasma.\textsuperscript{15} Unlike fHb and LDH, HI is not routinely displayed as a lab result since it is considered an internal parameter evaluating the preanalytical condition of blood samples but can be made readily available for clinicians free of charge.\textsuperscript{11–13} Previous investigations show good correlation of several quantitative and qualitative measurements of various analytical devices measuring HI to fHb concentrations in healthy volunteers or artificial plasma.\textsuperscript{14,16,17} Bosma et. al. investigated the potential of HI to be used as an alternative to fHb to detect hemolysis in patients on VV and VA ECMO.\textsuperscript{10}

We assessed the correlation of the automated hemolysis laboratory indicator HI (measured by the Roche Cobas C System, Roche Diagnostics International Ltd, Rotkreuz, Switzerland ) LDH and fHb in patients on various MCS to provide guidance for clinicians managing anticoagulation of patients with MCS and treating patients without ECLS suffering from hemolysis for autoimmune reasons or following transfusion of blood products.

We investigated a possible correlation of extreme values of fHb/HI with pump thrombosis or stoke in our patient collective and performed detailed cost analysis including fictional monthly costs of the different available laboratory parameters (fHb, LDH, HI) for timely detection of clot formation to address economic aspects of treatment.

**Methods**

We conducted a retrospective cohort study in our cardiothoracic ICU together with the cardiac surgical outpatient department managing patients on long term VAD support to determine whether HI or LDH can reliably and effectively used as an alternative to fHb as markers for hemolysis indirectly evaluating clot formation. Ethical committee approval was granted (EC number 1213/2019) and the need for informed consent was waived since all evaluated parameters were analysed as part of our best clinical practices in the ICU and the outpatient department. HI ≥ 20 has been correlated with mortality and pump clotting in a previous study\textsuperscript{10}, therefore measured fHb samples were first analyzed in their entirety and then stratified by HI above and below 20 to assess correlation of fHb, HI and LDH in the in all likelihood insignificant range of 0–19 and in the clinically more significant range above 20.

**Study population**
We included 96 patients with length of stay > 24h and age above 18 years who were treated in our cardiothoracic ICU between September and December 2018. 429 (40.7%) datasets consisting of simultaneous HI, LDH, fHb measurements were obtained from patients supported by an extracorporeal device in the ICU (ECMO/tRVAD/VADs with non-pulsatile flow or LVAD). 556 (52.8%) datasets were obtained from ICU patients without ECLS support while measurements were taken. Sixty-eight records (6.5%) assessed during routine outpatient follow-up visits of 51 LVAD patients older than 18 years were also included in our retrospective analysis. Out of 96 patients in the ICU, six patients received MCS (three by ECMO, one by LVAD and tRVAD, two by LVAD). The devices included in the ICU and outpatient LVAD groups were either HM2 (n = 7), HM3 (n = 24) or HVAD (n = 22) devices. One patient was supported by different devices during the ICU stay (initially on ECMO, than on LVAD plus tRVAD support), causing this to appear in both groups. Patients in the ICU group who received ECLS support before or after the study period were included in the group without ECLS support. For the cost analysis of a fictitious 10-bed ICU with 8 beds occupied by non-ECLS patients (screening for hemolysis once daily) and two ECLS patients (measurement of hemolysis parameters four times daily) and an occupancy rate of 100%, the monthly costs for fHb, LDH, and HI lab testing, respectively, were calculated.

Procedures and fHb and HI, LDH calculations

Blood was collected in siliconized vacuum tubes (Vacuette® 9NC Coagulation sodium citrate 3.2%, Greiner Bio-One, Kremsmuenster, Austria) for analysis at least once daily from patients in the ICU without (556 simultaneous measurements of fHb and HI and 481 simultaneous measurements of fHb and LDH), and multiple times daily from patients on ELCS (429 simultaneous measurements of fHb and HI and 399 simultaneous measurements of fHb and LDH) in order to detect hemolysis early in these patients. The analysis of the samples was then performed in the Central Laboratory of the Medical University of Vienna. fHb was measured in mg/dl by the reference pseudoperoxidase method 4-aminophenazone\(^{18}\). Before blood chemistry analysis, HI was measured by the Roche Cobas C 702 by taking a small portion of the patient's sample and after diluting it 0.9% sodium chloride solution assessing its absorbance of light at 570 and 600nm and then calculated \((1/40)*[(\text{Ext 600} - \text{Ext 570})-(122000* (\text{Ext 700} - \text{Ext 660}))]\). LDH was measured by determining its catalytic activity via reduction of NAD to NADH, and consecutive photometric measurement by Roche Cobas C from the same blood sample and the same vacuum tube as fHb and HI. Blood collection and analysis from the LVAD outpatients was performed in the same manner during regular outpatient follow-up visits resulting in 68 simultaneous measurements of fHb and HI and 67 simultaneous measurements of fHb and LDH from 51 patients. Descriptive data from ICU and outpatients were collected retrospectively. Cost analysis comparing the tests was performed by accessing information from the financial department.

Analysis Of Extreme Values Of Fhb/hi And Clinical Thrombotic Events
Electronic patient records of ICU patients on MCS and outpatients on LVAD support were investigated retrospectively for pump thrombosis, stroke or other thromboembolic events during the study period to assess a possible association with extreme values of HI > 20, HI > 50 and fHb > 50mg/dl. We chose these cutoff values based on previous investigations suggesting increased occurrence of circuit thrombosis in ECMO patients with HI above 20 and current ELSO guidelines regarding hemolysis with fHb above 50mg/dl as significant complications.\textsuperscript{10,19}

**Statistical analysis**

After preprocessing using Microsoft Excel, statistical analysis was performed using Python 3.9.2 with Pandas 1.4.1 and Scipy 1.8.0 package (Python Software Foundation, Fredericksburg, Virginia). Metric variables are described by mean and standard deviation or, in case of skewed distributions, by median [IQR]. Categorical variables are described by absolute and relative proportions. To analyze the association of fHb, LDH and HI in patients without and on ECMO or VAD support, correlation of the simultaneously acquired data was calculated by Pearson-correlation aiming for a correlation coefficient above 0.9 and \( p < 0.05 \). Statistical significance was set at \( p < 0.05 \).

**Results**

96 patients were treated in our ICU during the study period, median number of simultaneous HI, fHb, LDH measurements per patient on ECLS was 104 [19–128] and median number of simultaneous HI, fHb, LDH measurement on patients without ECLS was 4 [3–6]. In the 51 patients on LVAD support treated by the cardiac surgery outpatient clinic, median number of measurements per patient was 1 [1–1]. Baseline characteristics of patients are reported in table 1, correlations between fHb and HI and LDH are shown in Fig. 1 and table 2.

**HI and fHb**

Correlation between HI and fHb was significant in all patients \((r = 0.97, p < 0.01)\), ICU patients without ECLS \((r = 0.964, p < 0.01)\) as well as in ICU patients on ECLS \((r = 0.97, p < 0.01)\) and outpatients on LVAD support \((r = 0.827, p < 0.01)\). In the group of samples with HI below 20, we found an only moderate correlation of \(r = 0.799 \) \((p < 0.01)\) and excellent correlation of 0.955 \((p < 0.01)\) in the samples with HI \(\geq 20\). See table 2.

**LDH and fHb**

Correlation between LDH and fHb was low despite statistical significance in all patients \((r = 0.192, p < 0.01)\), ICU patients without ECLS \((r = 0.150, p < 0.01)\) as well as in patients on ECLS \((r = 0.161, p < 0.01)\) and was moderate in outpatients on LVAD \((r = 0.552, p < 0.01)\). See table 2.

Analysis of extreme values of fHb/Hi and clinical thrombotic events
Out of the 5 ICU patients with 6 ECLS devices, in all (100%) elevated HI > 20 was measured, median number of measurements HI > 20 was 2 (2–2). In 3 patients (60%) extreme values of HI > 50 and in 2 (40%) fHb > 50mg/dl were measured. One patient had 2 consecutive measurements of HI > 20 and 2 ICU patients had HI > 20 and one hat fHb > 50 on 2 consecutive days. No pump or oxygenator change hat to be performed in any patients. Analysis of electronic patient records and patient data management system records including flow, revolutions per minute, pulsatility index and power data from MCS during and after the respective period showed no hints of pump thrombosis. Out of the 51 LVAD patients, three measurements of HI > 20 and none with HI > 50 or fHb > 50mg/dl were obtained, none of them consecutively and no pump thrombosis or other thrombotic or thromboembolic events were recorded.

Cost analysis

The cost per fHb analysis was USD41.80, the cost per LDH was USD3.69, whereas the HI measurement did not incur any additional costs because this parameter is a routine internal preanalytical parameter. Median number of samples in the population of patients on ECLS in our ICU was 104 [19–128], therefore per ECLS patient in the ICU, cost of fHb was 4347.25 [794.21–5.350.46]USD and cost of LDH was 383.29 (70.02-471.74)USD. Total costs of fHb in all patients including LVAD outpatients was 41173.49USD, total cost of LDH in all patients was 3639USD while cost of HI was zero. In the fictional 10-bed ICU with 8 beds occupied by non ECLS patients and 2 ECLS patients, the monthly costs for fHb would be 20064.24USD and for LDH 1769.04USD, while HI monitoring would be free of charge. Detailed cost analysis is summarized in table 3.

Discussion

Our study results of excellent correlation between HI and fHb in patients on short and long-term MCS, confirm the previous positive results of Bosma et al., investigating the correlation of fHb and HI in patients on VV and VA ECMO using a Roche Cobas C 502 for HI and a DU 800 Spectrophotometer (Beckman Coulter, Brea, CA) for fHb analysis.10,14,16,17

Another major clinical finding is the excellent correlation between fHb and HI overall and in the subgroup of samples with a HI above 20. In the group of outpatients on LVAD support, correlation of fHb and HI was weaker (r = 0.827) but still significant (p < 0.01) which in all likelihood is due to a very low number of simultaneous measurements with HI above 20 in this subgroup. Previous findings in ECMO patients suggested increased occurrence of circuit thrombosis and possible negative impact on patient outcome in patients with HI above 20 while others found no such clear cutoff.1,10 Current ELSO guidelines do not recommend any fHb target, but regard hemolysis with fHb above 50mg/dl as significant complication, which is supported by investigations showing fHb above 50mg/dl in the early phase after ECLS implantation as an independent predictor of negative outcome.19,20 Hemolysis is defined in the INTERMACS registry as fHb > 20 mg/dl or serum LDH > 2,5 times the upper limit of the normal range21. Our investigations showed a very good correlation of HI and fHb in this range (above HI of 20, corresponding to a fHb of above 13 mg/dl in our cohort), confirming and elaborating the results of the
aforementioned previous investigation. This is encouraging since measurement results are very accurate in this subgroup where accuracy is of particular importance due to clinical impact. However, not only the exact target range may vary among different MCS systems, but also the accurate target range of HI may vary between different analytical devices since no overall standards exist.\textsuperscript{15}

Currently, results can be displayed quantitatively or as a percentage according to manufacturer and specific device performing the blood chemistry analysis and therefore HI measurement.\textsuperscript{14}

To tackle the problem in the future, we suggest either harmonization of HI calculation among all manufacturers and devices or displaying HI in a form resembling INR, namely HI-Ratio (HI-test/HI-normal). However, to make this possible, normal values for HI for each manufacturer and HI measurement device in very large groups of healthy patients have to be defined first.

We wanted to assess whether LDH can be used to assess the degree of hemolysis if neither fHb or HI are available or cannot be measured. One significant weakness of both fHb and HI is photospectroscopic analysis, therefore in situations with significant hyperbilirubinemia or hyperlipidemia, the serum's absorption of light is altered and measurement may be inaccurate.\textsuperscript{17} Unfortunately, hyperbilirubinemia is common in patients with ECLS due to hepatic dysfunction following acute or chronic RV failure and without ECLS suffering if hemolysis of immunological origin is excessive. LDH increase > 2.5 times above upper limit of normal range has been associated with pump thrombosis and thromboembolism in patients supported by HeartMate 2 and HeartWare VADs and is defined as a hemolytic adverse event by INTERMACS.\textsuperscript{9,10} Contrary to these findings and guidelines, LDH was no suitable replacement for HI or fHb in our investigation despite the high statistical significance of < 0.01 due to a clinically unacceptably low correlation index of only 0.192 with fHb in all patients. Performance was suboptimal in subgroups with or without ECLS or HI below or above 20, which we found an unprecise basis for significant therapeutic decisions. Of note, fHb and LDH correlated best in LVAD outpatients (r = 0.55). The most likely reason is that, other than the previous investigations, most measurements in our investigation correlating fHb with LDH were performed in critically ill patients. LDH is an enzyme existing in multiple cell types and serum levels rise in acute phase reactions as well as following lysis or necrosis not only of blood cells but of any cell in the body, therefore multiple reasons for LDH increases exist in patients after operative procedures or during critical illness.\textsuperscript{11–13}

Our cost analysis showed a relevant cost efficiency of HI over fHb in ICU patients on ECLS 4.347.25 (794.21-5350.46) USD per ECLS patient due to a median number of 104 measurements performed per patient. Thus, in a fictional 10 bed ICU with 2 patients being on ECLS (4 daily measurements) and 8 patients without ECLS support (1 daily measurements), preferring HI over fHb would save 20.064.24 USD per month and preferring HI over LDH measurements would save 1.769.04 USD per month. The amount saved by preferring HI over fHb is significant in a time of rising economic pressure on ICU clinicians and could instead be used to provide lifesaving treatment. The amount saved by preferring HI over LDH is far less significant, however due to the suboptimal correlation of HI with LDH in our cohort of patients treated
in the ICU, we discourage against the use of LDH as a marker for hemolysis in critically ill patients with MCS while acknowledging the role of LDH for monitoring outpatients on LVAD support.\(^3\)

There are limitations to our study. The number of simultaneous measurements of fHb, HI and LDH was satisfying in our investigation, but correlation of specific HI measurements or extreme values of HI > 50 or fHb > 50mg/dl with pump thrombosis or other thrombotic, thromboembolic or bleeding events was not possible in our collective unlike in investigations of other authors since adverse events were not the main focus of our retrospective study\(^10\). No such events as pump or oxygenator thrombosis occurred in our relatively small collective of 147 patients. One of our ICU patients may have suffered an ischemic stroke with hemorrhagic transformation during a window where a single measurement of HI > 20 occurred but the event cannot be matched to the exact period. Single measurements of elevated hemolysis parameters may be due to mishandled probes during or after blood withdrawal before analysis, or single measurements of increased hemolysis parameters may have led clinicians in our unit treating primarily patients before and after cardiac surgery and greatly experienced with ECMO and ECLS, to rapidly increase anticoagulation measures or perform other measures such as reducing ECLS flow to rapidly reduce hemolysis. Investigation of optimal HI/fHb ranges and cutoff values, as mentioned above, in large, prospective groups with different MCS devices is of great importance.

While the fact that only 47.2% of simultaneous fHb and HI measurements were taken from patients who were on ECLS when blood samples were taken can be seen as a weakness of our investigation, we emphasize that monitoring of hemolysis by HI can be essential in diseases such as hemolytic anemia for autoimmune reasons or following transfusion of blood products and the good correlation of HI with fHb we found may provide guidance for clinicians treating these patients.

We conclude that HI is favorable over fHb or LDH measurements in critically ill patients on MCS without extracorporeal life support to monitor for thrombosis due to its ready availability and advantageous cost factor. Further, prospective studies are needed to assess a possible role for HI in non-critically ill LVAD patients, the optimal therapeutic range of HI for each device, VV and VA ECMO, temporary RVAD and LVAD to minimize thrombosis due to over- and to minimize bleeding due to supra-therapeutic anticoagulation.

**Declarations**

Ethics approval and consent to participate

The Ethics Committee of the Medical University of Vienna and Vienna General Hospital approved the study (indicator number EK 1213/2019), the need for informed consent was waived due to the retrospective and observational nature of the study.

Data availability statement
The dataset used and analysed during the current study is available from the corresponding author on reasonable request.

Competing interests

Thomas Schloeglhofer is an Abbott and Medtronic consultant and received Abbott, Medtronic and CorWave research grants. None of the other authors has any financial relationship related to this article or other competing interests to disclose.

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Authors’ contributions

Bernhard Zapletal, Edda Tschernko, Martin Bernardi developed the concept of the study, Thomas Neugebauer, Michael Kainz, Monika Fritzer-Szekeres, Thomas Szekeres and Bernhard Zapletal collected the data and performed the statistical analysis. Bernhard Zapletal, Edda Tschernko, Daniel Zimpfer, Thomas Schloeglhofer, Martin Bernardi drafted the article. All authors performed critical revision of the article and approved the final version.

References


Tables

TABLE 1 baseline characteristics
Values are presented as number (n) and percentage (%), median [interquartile range]. Abbreviations: BMI, body mass index; ECLS, extra-corporeal life support; LVAD, left ventricular assist device; ICU, intensive care unit.

Table 2 is available in the Supplementary Files section.

TABLE 3 cost analysis

<table>
<thead>
<tr>
<th>n of measurements [Q1-Q3]</th>
<th>cost HI (USD)</th>
<th>cost fHb (USD)</th>
<th>cost LDH (USD)</th>
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</thead>
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<tr>
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<td>0</td>
<td>39.81</td>
</tr>
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<td>measurements ICU ECLS patients</td>
<td>104 [19-128]</td>
<td>0</td>
<td>4.347.25 (794.21-5350.46)</td>
</tr>
<tr>
<td>measurements ICU non-ECLS patients</td>
<td>4 [3-6]</td>
<td>0</td>
<td>167.20 (125.40-250.80)</td>
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<td>985</td>
<td>0</td>
<td>41173.49</td>
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<td>measurements per day ECLS patients*</td>
<td>4</td>
<td>0</td>
<td>167.20</td>
</tr>
<tr>
<td>measurements per day non-ECLS patients**</td>
<td>1</td>
<td>0</td>
<td>41.80</td>
</tr>
<tr>
<td>fictional 10 bed ICU per month (30d)***</td>
<td>480</td>
<td>0</td>
<td>20064.24</td>
</tr>
</tbody>
</table>

* suggested 6 hourly measurements of fHb/ HI/ LDH in ECLS patients, ** suggested 1 daily measurement of fHb/ HI/ LDH in non-ECLS patients, *** fictional 10 bed ICU being at 100% capacity with 2 patients being on ECLS support (4 daily measurements) and 8 patients without. Values are presented as number (n) and percentage (%), median [interquartile range]. Abbreviations: ECLS, extra-corporeal life support; ICU, intensive care unit.
Figure 1

A) correlation diagram of fHb and HI measurements in all patients, B) correlation diagram of fHb and HI measurements with HI<20 in all patients, C) correlation diagram of fHb and HI, measurements with HI≥20 in all patients, D) correlation of fHb and HI in LVAD outpatients E) correlation of fHb and LDH in LVAD outpatients. Incomplete pairs of measurements (as in table 2), are not displayed F) correlation of fHb and LDH in all patients. Incomplete pairs of measurements (as in table 2) are not displayed
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table2.jpg