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Monocytic human leukocyte antigen DR expression in young infants undergoing cardiopulmonary bypass

Running Head: mHLA-DR and pediatric cardiac surgery

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Abstract

Background: Monocytic human leukocyte antigen DR (mHLA-DR) expression levels have been reported to be a marker of immunosuppression and a predictor of sepsis and mortality. There is, however, scant data regarding mHLA-DR monitoring in young infants after cardiopulmonary bypass. Our objectives were to investigate the kinetics of mHLA-DR expression and to determine whether mHLA-DR levels are associated with healthcare-associated infection (HAI) after cardiopulmonary bypass in young infants.

Methods: mHLA-DR levels were analyzed by flow cytometry using a standardized method in 49 infants (< 3 months old) with congenital heart disease before and after cardiopulmonary bypass. Results are expressed as the number of anti-HLA-DR antibodies per cell (AB/c).

Results: Postoperative mHLA-DR expression was reduced in all of the infants. Eleven patients (22%) developed HAI and 4 patients (8%) died during the 30-day follow-up. mHLA-DR expression was significantly lower on postoperative day 4 in the HAI group compared to those who without HAI (3,768 AB/c [1,938-6,144] vs. 13,230 AB/c [6,152-19,130]; $p = 0.0142$). Although mHLA-DR expression is associated with postoperative severity, $mHLA-DR \leq 4,500$ AB/c in first 72 hours among patients with higher postoperative severity (extracorporeal membrane oxygenation and/or corticoids and/or delayed closure of sternum) is associated with occurrence of HAI in univariate analysis (OR = 6.3, 95% CI 1.0; 38.7; $p = 0.037$).

Conclusions: Cardiopulmonary bypass induces a profound decrease in mHLA-DR expression in young infants. Among patients with higher postoperative severity, low level of mHLA-DR in the early postoperative period is associated with the development of HAI.

Expression of human leukocyte antigen-DR on monocyte cell surface (mHLA-DR) is one of the immunological assays used to measure anti-inflammatory response to a physiologic stressor. Reduced mHLA-DR expression reflects a state of immunosuppression. When this is severe and persistent, it is referred to as “immunoparalysis” [1]. To date, mHLA-DR expression has been assessed as a predictor of sepsis and mortality after various injuries in adults as well as in children [2-5]. The predictive value of this marker has also been investigated after pediatric cardiac surgery with cardiopulmonary bypass (CPB), irrespective of age [6, 7]. As a younger age is a well-known risk factor of postoperative complications [8] and, as suggested by our team in a pilot study [9], we hypothesized that mHLA-DR expression levels may provide further valuable information regarding the risk of sepsis in young infants after CPB. Thus, our objectives were to investigate the kinetics of mHLA-DR expression before and after CPB and to determine whether mHLA-DR levels are associated with healthcare-associated infection (HAI) within 30 days after CPB in young infants.

PATIENTS AND METHODS

Study design

This was a prospective observational study in infants younger than 3 months of age who underwent cardiac surgery with CPB between February 2018 and March 2019. Exclusion criteria were a known immune deficiency and surgery outside the normal workweek.

Ethics approval

This study was approved by the Institutional Review Board for ethics (IRB 2017-A02845-48) and was carried out in accordance with the recommendations of the commission for informatics and freedom MR003.

Data collection

The primary outcome was the occurrence of HAI. HAI, including bloodstream infections, pneumonia, sternal wound infections, and urinary tract infections, were prospectively recorded within 30 days after the surgery. The physicians were not informed of the mHLA-DR results. All of the infants received

antibiotic prophylaxis with cefuroxime for 48 hours. All of the patients treated with antibiotics (other than prophylaxis) and/or with bacterial identification during the 30-day follow-up were retrospectively reviewed by three physicians blind of the mHLA-DR results (AC, PB, and NJ). The final diagnosis of HAI was established according to the international guidelines [10]. We defined death as a patient dying within 30 days of the surgery. No steroid was given at our institution prior cardiopulmonary bypass. However, hydrocortisone (2 mg/kg every 6 hours) may be administered at physician discretion in case of low cardiac output syndrome and tapered over 2 days.

Blood collection and measurement of mHLA-DR levels by flow cytometry

Venous EDTA anti-coagulated samples were collected at four time points. The first sample was obtained in the operating room prior to the onset of CPB. Three other samples were collected during the first seven postoperative days (day 2 \pm 1, day 4 \pm 1, and day 6 or 7) according on Immunology Laboratory working days (Monday to Friday). The number of HLA-DR molecules per monocyte (AB/c) was determined immediately after sample collection (i.e. within 90 min) by flow cytometry on fresh whole blood using a standardized method as previously described [11, 12]. The clinical data were not available to the immunology staff.

Statistical analysis

The baseline characteristics are reported as the median [interquartile range] and n (%) for the quantitative and qualitative variables, respectively. mHLA-DR levels were expressed as the number of anti-HLA-DR antibodies per cell (AB/c) and were presented by median and interquartile range. Comparison of mHLA-DR expression at different time points was performed using the Kruskal-Wallis test with Dunn's multiple comparison test. The patients were separated into two groups according to the occurrence of HAI. The quantitative variables were compared using the Mann-Whitney test and the qualitative variables were compared using Pearson's Chi-square test. Furthermore, we investigated the kinetics of mHLA-DR expression before and after CPB according to different variables related to postoperative severity (postoperative extracorporeal membrane oxygenation (ECMO), use of steroids during the first 72h and delayed closure of sternum). Finally, as we noted a variation in blood

sampling related to working and non-working days, lowest value of mHLA-DR in first 72 hours after CPB was studied. The potential of lowest mHLA-DR in first 72 hours to predict HAI was assessed with receiver operating characteristic (ROC) curve. The cut-off value was determined by maximizing the Youden index, which is the sum of sensitivity and specificity -1. Univariate analyses were performed to assess the association between postoperative factors and lowest mHLA-DR expression in first 72 hours with the occurrence of HAI.

RESULTS

Clinical characteristics of the patients

Forty-nine infants were analysed, including 28 neonates (57%), with a median age of 14 days [7-63]. Congenital heart diseases included transposition of the great arteries (n = 19, of which 7 with ventricular septal defect and 2 with coarctation of the aorta), aortic valve disease (n = 8), ventricular septal defect (n = 7), aortic arch hypoplasia (n = 4), tetralogy of Fallot (n = 4), complete atrioventricular canal (n = 4), aortopulmonary window (n = 1), pulmonary atresia (n = 1) and double outlet right ventricle (n = 1). Only 1 patient required intubation and 3 had an inotropic support (milrinone) preoperatively. Eleven infants (24%) developed HAI with a median delay of 7 days [5-14]: sternal wound infection (n = 6), pneumonia (n = 2), urinary tract infection (n = 2), and bloodstream infection (n = 1). Table 1 shows univariable comparison of demographics and clinical outcomes according to occurrence of HAI. As expected, patients in HAI group had longer CPB duration and differed largely in term of characteristics during the PICU stay. Four patients died at day 6, 7, 10, and 11 after the surgery, of whom one had developed HAI (See Supplemental Table 1 for details concerning deceased infants).

mHLA-DR expression before and within 7 days after CPB

In the overall patient population, mHLA-DR expression was significantly reduced after CPB compared with the preoperative level, with the lowest level of expression observed at day 1 (25,031 AB/c [18,654-35,282] vs. 4,624 AB/c [3,142-7,779]; $p < 0.001$) and without recovery at day 6-7 (25,031 AB/c [18,654-35,282] vs. 11,904 AB/c [6,912-16,217]; $p < 0.001$) (Figure 1). Although

mHLA-DR expression was lower in neonates compared to older infants preoperatively (21,538 AB/c [12,713-30,170] vs. 29,344 AB/c [24,702-41,866]; $p = 0.002$), no difference was observed after CPB (data not shown).

Comparison of the mHLA-DR expression according to the occurrence of HAI is shown in Figure 2. mHLA-DR expression was significantly lower at day 4 in the HAI group compared to infants without HAI (3,768 AB/c [1,938-6,144] vs. 13,230 AB/c [6,152-19,130]; $p = 0.0142$).

mHLA-DR expression in relation to postoperative illness severity

The association between HAI and low values of mHLA-DR could reflect interdependence between mHLA-DR and postoperative illness severity. Thus, we investigated the kinetics of mHLA-DR expression before and after CPB according to different variables related to the severity of the insult (postoperative ECMO, use of steroids during the first 72h and delayed closure of sternum). Interestingly, mHLA-DR expression was lower within 3 days after CPB in patients with higher severity compared to others, suggesting an association between mHLA-DR expression and postoperative illness severity (Figure 3).

mHLA-DR expression in first 72 hours after CPB in relation to HAI

Lowest mHLA-DR in first 72 hours was 3,894 AB/c [2,413-6,491] in HAI group compared to 5,665 [3,670-7,569] in the non-HAI group ($p = 0.141$). The lowest mHLA-DR level in first 72 hours that gave the best specificity and sensitivity to predict HAI calculated after the Youden index was 4,500 AB/c. Univariate analyses were performed to assess the association between postoperative factors and mHLA-DR expression $\leq 4,500$ AB/c in first 72 hours with the occurrence of HAI (Table 2). Interestingly, among 28 patients with higher postoperative severity (ECMO and/or corticoids and/or delayed closure of sternum), 10 developed HAI and mHLA-DR expression $\leq 4,500$ AB/c in first 72 hours was associated with occurrence of HAI in univariate analysis (OR = 6.3, 95% CI 1.0; 38.7; $p = 0.037$) (Figure 4).

COMMENT

The impact of CBP on mHLA-DR expression has already been explored in children, with a substantial degree of heterogeneity regarding the age of the patients. Here, we focused on young infants, as a young age is a well-known risk factor of HAI after paediatric cardiac surgery [8]. It is important to note however that some congenital heart disease repair (e.g., complete common atrioventricular canal, ventricular septal defect) can be performed at any age, with increased risk associated with smaller patients size. Institutional practices differ among centers, and may influence our cohort characteristics and outcome [13].

Here, we observed a profound decrease of mHLA-DR measurement after CBP in young infants, reflecting severe immune suppression. Although other measures of immune system function need to be explored, at least one innate immune cell-line is earlier impact by CPB in our cohort. The factors involved in the development of severe immunoparalysis earlier after CPB are not fully understood [1]. Earlier immunological pathways could be involved in the decrease of mHLA-DR expression. For example, a release of danger-associated molecular patterns (DAMPs) in a very early phase after trauma is associated with the extent of immune suppression, as indicated by decrease of HLA-DRA gene expression [14]. Moreover, Le Tulzo *et al.* reported a negative correlation between cortisol levels and mHLA-DR expression during septic shock *in vivo*, with a down modulation effect of corticosteroids on HLA-DR expression *in vitro* [15]. As we noted an association between mHLA-DR and postoperative severity, focusing on patients with higher postoperative severity seems to be crucial to further decipher mechanisms leading to the decrease of mHLA-DR after CPB.

Expression of mHLA-DR was significantly lower on postoperative day 4 in the HAI group compared to infants without HAI. This result could however reflect interdependence between mHLA-DR level and postoperative illness severity. It is well established that some postoperative factors (e.g., delayed closure of the sternum, postoperative ECMO, use of corticoids) are independently associated with HAI [8, 16] and we observed an association between mHLA-DR expression and postoperative severity. Thus, we focused on infants with higher postoperative severity, defined by the use of ECMO and/or corticoids and/or delayed closure of sternum, and reported an interesting association between mHLA-DR expression in first 72 hours after CPB and occurrence of HAI. This result is consistent with

previous studies in older children requiring CPB [6, 7]. Although this association is not a proof of causative relationship, a value less than 4,500 AB/c earlier after CPB seems to be an interesting threshold to predict infection earlier after CPB among infants with higher postoperative severity.

Our study has several limitations. Because of the small study number it is impossible to assert causality in the relationship between the sustained reduction in mHLA-DR and the development of HAI. Several differences were noted between HAI and no HAI group. For example, need for ECMO, use of postoperative steroids and delayed sternal closure are important confounders to immune response which need to be specifically addressed in further studies [17, 18]. Only large multicenter studies would allow us to adequately determine whether mHLA-DR expression is independent of the others factors associated with HAI after CBP. Moreover, regarding the Society of Thoracic Surgeons (STS) predicted mortality [19], our mortality rate is likely high in our population. All deceased patients received ECMO support after CPB and three out of 4 had residual lesions. Residual lesions are present approximately one-quarter of postoperative cardiac surgery requiring ECMO support, justifying earlier detection of residual lesions and reintervention in order to improve clinical outcome [20]. Finally, the timing of the mHLA-DR measurements was not standardized, which is inherent to the use of a flow cytometry facility that is only available on weekdays. Access to an automated bedside flow cytometer could help overcome this issue [21].

Conclusion

Cardiac surgery requiring CPB in young infants is associated with significant reduction in mHLA-DR expression, suggesting that at least one innate immune cell-line is impacted by CPB. Among infants with higher postoperative severity, a profound reduction of mHLA-DR expression earlier after CPB is associated with HAI in our series and large multicenter studies are needed to determine whether mHLA-DR expression is independent of the others factors associated with HAI. Further investigations may help clinicians to propose targeted interventions, such as antibiotic prophylaxis or immunostimulatory therapy in order to reduce the risk of HAI after CBP in infants with higher postoperative severity.

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Table 1: The characteristics of the infants (n = 49)

Variables	Infants with HAI (n=11)	Infants without HAI (n=38)	p-value
Demographics			
Age at the time of surgery (days)	25 [8-59]	12 [7-63]	0.494
Neonate (age < 28 days of life), n (%)	6 (55%)	22 (58%)	0.843
Birth weight (kg)	3.2 [2.7-3.7] n=9	3.3 [2.7-3.6] n=37	0.935
Male, n (%)	9 (82%)	24 (63%)	0.245
Gestational age < 36 weeks, n (%)	1 (9%)	2 (5%)	0.641
Genetic abnormality, n (%)	3 (27%)	3 (8%)	0.084
Cyanotic congenital heart defects, n (%)	5 (45%)	16 (42%)	0.843
Preoperative intubation, n (%)	1 (9%)	0 (0%)	0.060
Preoperative inotropic support, n (%)	1 (9%)	2 (5%)	0.641
Preoperative lactate level (mmol/L)	1.2 [1.1-1.7] n=10	1.5 [1.3-2.1] n=35	0.099
Preoperative lymphocyte cell count (/mm ³)	3,960 [3,230-5,900]	4,400 [3,285-6,080] n=37	0.677
Characteristics of the surgery			
RACHS-1 score	3 [3-4]	3 [2-4]	0.370
Cardiopulmonary bypass time (min)	209 [194-271]	133 [82-195]	0.011
Aortic cross-clamp time (min)	134 [92-175]	70 [46-105]	0.014
Selective cerebral perfusion, n (%)	2 (18%)	4 (11%)	0.495
Hypothermia (≤ 28 °C), n (%)	3 (27%)	4 (11%)	0.162
Pre- or intraoperative use of steroids	0 (0%)	0 (0%)	1.000
Characteristics during the PICU stay			
Use of hydrocortisone (2 mg/kg every 6 hours during the first 72 h, n (%))	9 (82%)	18 (47%)	0.043
Extracorporeal membrane oxygenation, n (%)	3 (27%)	3 (8%)	0.084
Delayed closure of the sternum, n (%)	8 (73%)	11 (29%)	0.009
Peak VIS during the first 72 h ^a	17 [12-23]	10 [9-13]	0.044
LCOS, n (%) ^b	6 (55%)	6 (16%)	0.008
Positive fluid balance at day 1, n (%)	6 (55%)	17 (45%)	0.566
Need for dialysis, n (%)	1 (9%)	0 (0%)	0.006
Median total volume of transfusion during the first 72 h (ml/kg)	16 [0-68]	12 [0-34]	0.193
Minimal lymphocyte cell count during the first 72 h (/mm ³)	960 [630-1500]	1,475 [918-2100]	0.089

Duration of mechanical ventilation (days)	8 [2-10]	2 [1-4]	0.007
PICU length of stay (days)	13 [7-21]	6 [4-7]	0.001
Death, n (%)	1 (9%)	3 (8%)	0.898

The data are expressed as medians (interquartile ranges) or n (%).

^a VIS = Vasoactive-Inotropic score

^b LCOS = Low Cardiac Output Syndrome, defined by a maximal VIS during the first 72 h \geq 20 and/or a need for postoperative ECMO support

HAI: healthcare-associated infection; PICU: pediatric intensive care unit

Table 2: Bivariate analysis of variables related to the severity and lowest mHLA-DR expression in first 72 hours with the occurrence of HAI

Variables			Univariate		
			OR	95% CI	p
ECMO	Positive	n = 6	4.4	0.7; 25.8	0.084
Use of corticosteroids during the first 72 h	Positive	n = 27	5.0	1.0; 26.3	0.043
Delayed closure of sternum	Positive	n = 19	6.5	1.5; 29.4	0.009
Lowest mHLA-DR in first 72 h	≤ 4500	n = 19	6.5	1.5; 29.4	0.009

Figure Legends

Figure 1: Time course of monocyte human leukocyte antigen-DR (mHLA-DR) expression in all patients (n=49) after cardiopulmonary bypass. The number of HLA-DR molecules per monocyte (AB/c) was determined by flow cytometry on fresh whole blood preoperatively and during the first seven postoperative days (preoperatively, n =49; D1, n = 9; D2, n = 34; D3, n = 17; D4, n = 26; D5, n = 12; D6-7, n = 39). The data are shown in box-and-whisker plots. The upper and lower borders of the box represent the 75th and 25th quartiles, the middle horizontal line represents the median and the upper and lower whiskers represent the maximum and minimum values of non-outliers. Outliers are shown as symbols. Comparison of the mHLA-DR expression at different time points was performed using the Kruskal-Wallis test with Dunn's multiple comparison test (* p < 0.05; **p < 0.01; *** p < 0.001). Of note, mHLA-DR measurements were not available at D6-7 for 10 infants including one death at day 6.

Figure 2. mHLA-DR expression levels after cardiopulmonary bypass according to the occurrence of HAI. The number of HLA-DR molecules per monocyte (AB/c) was determined by flow cytometry on fresh whole blood preoperatively and during the first seven postoperative days. As mHLA-DR expression is known to be reduced in septic patients, the values were censored once infection occurred for 5 patients at day 6-7. Individual data points are represented as well as the median and interquartile range according to the occurrence of healthcare-associated infection (HAI) . (red box: HAI group with n = 11 preoperatively, n = 2 at D1, n = 8 at D2, n = 6 at D3, n = 4 at D4, n = 2 at D5 and n = 7 at D6-7; blue box: non HAI group with n = 38 preoperatively, n = 7 at D1, n = 26 at D2, n = 11 at D3, n = 22 at D4, n = 10 at D5 and n = 26 at D6-7). The Mann–Whitney test was used to compare the two groups of infants (*p < 0.05).

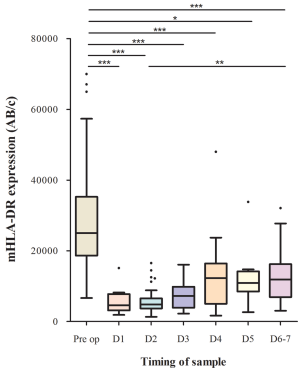
HAI: healthcare-associated infection

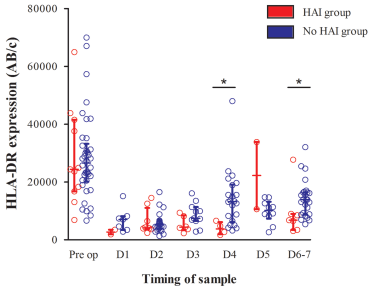
Figure 3. mHLA-DR expression in relation to postoperative illness severity. The number of HLA-DR molecules per monocyte (AB/c) was determined by flow cytometry on fresh whole blood preoperatively and during the first seven postoperative days. Individual data points are represented as

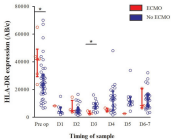
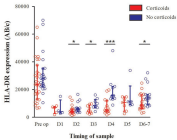
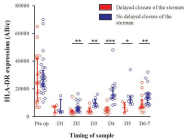
well as the median and interquartile range according to different variables related to the severity of the insult: (A) postoperative ECMO, (B) use of steroids during the first 72 hours and (C) delayed closure of sternum.

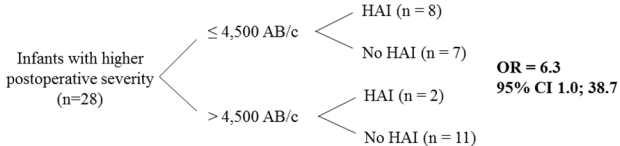
Figure 4. Relationship between the lowest mHLA-DR in first 72 hours and occurrence of HAI among patients with higher postoperative severity. Flow chart of the number of subjects classified with or without HAI according to lowest mHLA-DR expression in first 72 hours. Higher postoperative severity is defined by need of postoperative ECMO and/or use of corticoids during the first 72 hours and/or delayed closure of sternum (n = 28).

HAI: healthcare-associated infection





A**B****C**



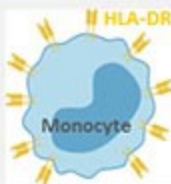
Monocytic human leukocyte antigen DR expression in young infants undergoing cardiopulmonary bypass



49 infants < 3 months with congenital heart disease requiring cardiopulmonary bypass were included in this prospective single-center study.



Blood samples were collected prior to the onset of cardiopulmonary bypass and within 7 days after surgery. The number of HLA-DR molecules per monocyte was determined by flow cytometry.



- Postoperative mHLA-DR expression was reduced in all of the infants.
- mHLA-DR expression was significantly lower on postoperative day 4 in the infected group compared to non infected group ($p = 0.014$).
- mHLA-DR expression is associated with postoperative severity.
- $mHLA-DR \leq 4,500$ AB/c in first 72h among patients with higher postoperative severity is associated with occurrence of infection (OR = 6.3, 95% CI 1.0; 38.7; $p = 0.037$).

Among patients with higher postoperative severity, mHLA-DR $\leq 4,500$ AB/c in the early postoperative period is associated with occurrence of infection. Further investigations may help clinicians to propose targeted interventions in order to reduce the infectious risk in this population.

