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Multicenter inter-laboratory quality control of monocyte HLA-DR expression by flow cytometry

To the Editor

After injury, the onset of severe immunosuppression among patients in intensive care units (ICUs) is a well-documented phenomenon (Cajander et al., 2024). Hence, there is an increasing hypothesis that restoring immune function in ICU patients could provide a therapeutic avenue, potentially utilizing agents such as IFN- γ , GM-CSF, or IL-7. That given, in the era of personalized medicine, a critical aspect revolves around our ability to identify the most immunosuppressed patients (i.e., those who stand to benefit the most from immunostimulating therapies). This becomes even more pertinent as randomized controlled trials (RCTs) in the field are now conducted in a multicenter fashion.

To date, diminished expression of human leukocyte antigen-DR on circulating monocytes (mHLA-DR), measured by flow cytometry, is widely acknowledged as a reliable indicator of immunosuppression in critically ill patients (Cajander et al., 2024). Consequently, mHLA-DR assessment is used for patient stratification in RCT. In the context of the multicenter IGNORANT study (NCT05843786), which evaluates the adjunctive use of IFN- γ in ventilator-acquired pneumonia (VAP) among immunosuppressed ICU patients, a prerequisite was to check mHLA-DR measurements to ensure reproducible results across centers. Although not strictly mandatory, conducting such an inter-

laboratory control was considered a pertinent precaution for a study where mHLA-DR serves as an inclusion criterion.

To achieve this, we conducted an inter-laboratory control involving nine centers across France. We utilized stabilized blood samples ($n = 3$), commonly employed for routine lymphocyte counts and quality control. These samples contain monocytes with a measurable level of mHLA-DR (but not included in manufacturers specifications), which were blindly tested across all participating centers. Staining procedures and measurements adhered to a standardized protocol (see details in Demaret et al., 2013; Docke et al., 2005; Quadri et al., 2021). Results, calculated from the median of fluorescence of the entire monocyte population using calibrated beads, were reported as the number of antibodies bound per cell (AB/C). Cell acquisition was carried out using four types of flow cytometers from diverse manufacturers (refer to Table 1 and Table S1). Each center analyzed each sample in duplicate. Median, standard deviation (SD), and coefficient of variation (CV) were calculated for each sample using all values from all centers. Z-scores were then computed for each measurement based on the mean and SD. The complete results are presented in Table 1.

The main finding of this report is the excellent results observed. All CV remained below 9.2%, with all Z-scores falling

TABLE 1 mHLA-DR inter-laboratory assessment. mHLA-DR was assessed in duplicate (#1 and #2) at each of the nine centers (with two centers employing two different flow cytometers) using three samples of stabilized blood from the same lot (A = Multicheck, Becton-Dickinson, lot: BM034N, B = Multicheck Low, Becton-Dickinson, lot: BM3024L, C = Immunotrol, Beckman-Coulter, lot: 7587289). SD = standard deviation, CV = coefficient of variation. Z-score = (value-mean)/SD. BD = Becton-Dickinson, BC = Beckman-Coulter.

Center	Cytometer	Sample	#1 (AB/C)	#2 (AB/C)	Mean (AB/C)	SD (AB/C)	Z-score	CV (%)
1	Navios 1-BC	A	34,082	34,082	32,461	2270	0.7	7.0
1	Navios 2-BC	A	31,136	30,030			-0.8	
2	DXFlex-BC	A	34,709	32,967			0.6	
3	FACS Lyric-BD	A	31,743	31,606			-0.3	
4	DXFlex-BC	A	35,801	35,155			1.3	
5	FACS Canto II-BD	A	32,811	33,066			0.2	
6	DXFlex-BC	A	34,771	34,498			1	
7	DXFlex-BC	A	34,459	32,683			0.5	
8	Navios-BC	A	32,933	30,819			-0.3	
9	FACS Canto II-BD	A	28,395	26,559			-2.2	
9	FACS Lyric-BD	A	31,989	29,846			-0.7	

(Continues)

TABLE 1 (Continued)

Center	Cytometer	Sample	#1 (AB/C)	#2 (AB/C)	Mean (AB/C)	SD (AB/C)	Z-score	CV (%)
1	Navios 1-BC	B	26,332	29,960	25,506	2247	1.2	8.8
1	Navios 2-BC	B	23,443	23,883			-0.8	
2	DXFlex-BC	B	29,500	29,334			1.7	
3	FACS Lyric-BD	B	25,690	24,499			-0.2	
4	DXFlex-BC	B	26,340	25,929			0.3	
5	FACS Canto II-BD	B	24,190	23,935			-0.6	
6	DXFlex-BC	B	27,397	26,532			0.6	
7	DXFlex-BC	B	25,420	24,629			-0.2	
8	Navios-BC	B	26,938	25,433			0.3	
9	FACS Canto II-BD	B	21,107	21,252			-1.9	
9	FACS Lyric-BD	B	25,523	23,867			-0.4	
1	Navios 1-BC	C	21,726	22,135	19,976	1846	1.1	9.2
1	Navios 2-BC	C	19,876	20,616			0.1	
2	DXFlex-BC	C	22,256	22,089			1.2	
3	FACS Lyric-BD	C	17,824	18,586			-1	
4	DXFlex-BC	C	19,858	19,749			-0.1	
5	FACS Canto II-BD	C	20,050	18,808			-0.3	
6	DXFlex-BC	C	22,825	21,527			1.2	
7	DXFlex-BC	C	22,492	20,449			0.8	
8	Navios-BC	C	19,235	19,312			-0.4	
9	FACS Canto II-BD	C	16,194	16,735			-1.9	
9	FACS Lyric-BD	C	18,503	18,621			-0.8	

within the accepted range of $[-3/+3]$. Notably, only a single value fell between 2 and 3, signaling caution, yet remaining within acceptable parameters. Collectively, these findings are remarkable, especially given the use of a manual flow cytometry protocol and the calculation of results based on median fluorescence intensities, indicative of a meticulously standardized procedure. These results allow us to confidently anticipate the inclusion of patients in future studies that will strictly adhere to the present standardized protocol for measuring mHLA-DR.

To our knowledge, this is the first inter-laboratory control of this scale, encompassing nine centers. Previous comparisons between laboratories were limited to two centers, which demonstrated good correlation (Demaret et al., 2013; Quadrini et al., 2021) or three centers showing coefficient of variations (CVs) between 15% and 25% in 2005. The latter marked the inaugural publication of the standardized protocol utilized in this study (Docke et al., 2005). After years of implementation across various centers, on the basis of the present results, we confidently assert that this protocol is now primed for routine use and RCT.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All raw data are available in Table 1.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.