Profiling Class I and II Anti-HLA Antibody Reactivity in Unsensitized Men Using Recent Lots ofthe Luminex Single Antigen Beads Assay

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INTRODUCTION

Previous studies have shown that single antigen bead (SAB) assays frequently detect anti-HLA reactivity in healthy, nonalloimmunized men[1]. Subsequent investigations have shown that some of these naturally occurring antibodies recognize cryptic epitopes exposed on denatured HLA molecules rather than native HLA expressed on the cell surface. Such reactivity may lead to false-positive donor-specific antibody assignments, thereby compromising the accuracy of virtual crossmatching and potentially resulting in the unnecessary exclusion of suitable donors. Therefore, the present study aimed to profile anti-HLA antibody reactivity in men without sensitizing events using recent lots of the Luminex SAB assay.

METHODOLOGY

Assessing the MARCo platform (*marco.igen.org.br*), we identified 4,004 unsensitized men, of whom 2,649 and 1,355 had available Class I SAB and Class II SAB results, respectively. The five most recent Class I and II SAB lots from Thermo Fisher were evaluated, including lots 11–15 for Class I and 13–17 for Class II. The cutoff for SAB positivity was MFI≥1500. For positive reactions, MFI values were summarized as median and interquartile range (IQR). We included the five most common SAB reactions for each locus in our local cPRA tool (*https://www.igen.org.br/calculadorapra18ws*) to assess the overall impact on the transplantability of these patients.

RESULTS

In total, there were reactivity against HLA-A, -B, -C, -DR, DQB1/DQA1, and DPB1/DPA1 antigens in 159 (6.0%), 113 (4.3%), 73 (2.7%), 66 (4.8%), 171 (12.6%), and 57 (4.2%) of the unsensitized patients, respectively. Overall, the median MFI and IQR for each HLA locus are as follows: HLA-A, median=5,525, IQR=4,982−6,570; HLA-B, median=5,441, IQR=4,604−5,966; HLA-C, median=4,171, IQR=3,736−4,542; HLA-DR, median=6,848, IQR=5,845−8,045; HLA-DQA1/DQB1, median=12,283, IQR=11,086−13,661; and HLA-DPA1/DPB1, median=10,735, IQR=8,405−12,338. The ten most common reactions for HLA-A, -B, -C, -DR,DQB1/DQA1, and DPB1/DPA1, along with their median and IQR MFI values, are shown in Figure 1, while Figure 2 highlights the top ten Class I and II reactions across individual SAB lots. Additionally, we observed that including the five most common SAB reactions for HLA-A, -B, -C, -DR, DQB1/DQA1, and DPB1/DPA1 would yield cPRAs of 32.3%, 28.1%, 60.1%, 46.2%, 67.1%, and 54.6%.

Figure 1. Top ten SAB reactions for HLA-A, -B, -C, -DR, DQB1/DQA1, and DPB1/DPA1 in unsensitized men

HLA-A			HLA-B			HLA-C		
Allele	Positive (%)	Median MFI (IQR)	Allele	Positive (%)	Median MFI (IQR)	Allele	Positive (%)	Median MFI (IQR)
A*25:01	8.42	4754 (2392–13491)	B*15:12	11.51	4456 (2498–9309)	C*17:01	4.57	3870 (2325–10054)
A*24:02	8.27	4897 (2450–13608)	B*57:01	9.74	4188 (2503–9794)	C*02:02	3.4	4701 (2724–12557)
A*23:01	7.81	5281 (2700–13441)	B*57:03	8.87	4608 (2617–10417)	C*05:01	3.21	5292 (2842–16294)
A*66:01	7.51	5525 (2814–12732)	B*45:01	8.57	5002 (2682–11557)	C*15:02	3.17	5942 (2497–14901)
A*24:03	7.4	5180 (2618–14883)	B*44:03	7.44	5115 (2413–13183)	C*04:01	3.1	3605 (2065–7676)
A*02:01	6.8	7514 (3076–18500)	B*44:02	7.4	5508 (2658–12514)	C*18:02	3.1	4187 (2606–12897)
A*66:02	6.61	4617 (2559–12010)	B*58:01	7.25	4476 (2484–10875)	C*06:02	2.98	4489 (2598–12684)
A*69:01	6.53	5855 (2804–15084)	B*82:01	6.64	4507 (2485–12312)	C*03:03	2.76	4297 (2188–7836)
A*26:01	6.49	5111 (2786–11718)	B*15:16	6.42	4299 (2565–10087)	C*01:02	2.72	3884 (2080–6792)
A*02:03	6.42	7366 (3274–18149)	B*08:01	6.34	4603 (2381–10101)	C*12:03	2.72	3483 (2212–6567)

HLA-DR			HLA-DQB1/DQA1			HLA-DPB1/DPA1		
Allele	Positive (%)	Median MFI (IQR)	Allele	Positive (%)	Median MFI (IQR)	Allele	Positive (%)	Median MFI (IQR)
DRB1*09:01	7.75	5859 (3241–16937)	DQB1*03:02 / DQA1*03:03	21.6	12283 (5515–19050)	DPB1*01:01 / DPA1*02:01	9.67	2907 (2084–9010)
DRB1*09:02	7.53	7484 (3216–16843)	DQB1*03:02 / DQA1*03:02	19.59	11336 (4893–18903)	DPB1*14:01 / DPA1*02:01	5.61	11933 (3554–19495)
DRB4*01:01	7.16	8502 (2851–20393)	DQB1*03:03 / DQA1*03:02	19.34	12020 (4843–19699)	DPB1*09:01 / DPA1*02:01	5.54	12405 (4267–20442)
DRB4*01:03	7.16	9587 (3180–20229)	DQB1*03:19 / DQA1*05:05	18.75	13249 (6266–19026)	DPB1*17:01 / DPA1*02:01	5.46	12394 (4693–19674)
DRB1*07:01	6.64	5564 (2788–14135)	DQB1*03:01 / DQA1*06:01	18.38	13016 (5749–18853)	DPB1*06:01 / DPA1*01:03	5.31	11232 (5259–19193)
DRB1*04:01	6.49	5306 (2437–12106)	DQB1*03:01 / DQA1*05:03	18.08	13661 (6370–20155)	DPB1*20:01 / DPA1*03:01	5.24	11518 (6095–19043)
DRB1*04:04	6.27	5406 (2363–12874)	DQB1*03:03 / DQA1*02:01	17.86	11187 (4121–20086)	DPB1*10:01 / DPA1*02:02	5.02	11227 (3029–19103)
DRB1*10:01	6.05	9326 (3753–18196)	DQB1*03:03 / DQA1*03:01	17.64	10405 (4391–20445)	DPB1*03:01 / DPA1*01:03	4.94	12585 (6437–19353)
DRB1*14:01	6.05	5806 (3037–14934)	DQB1*03:02 / DQA1*03:01	17.42	10772 (4855–21072)	DPB1*06:01 / DPA1*02:01	4.94	12868 (5829–20591)
DRB5*01:01	5.98	8012 (2724–16036)	DQB1*03:02 / DQA1*02:01	16.46	10988 (4231–20401)	DPB1*03:01 / DPA1*01:05	4.87	13025 (6130–19859)

Figure 2: Top ten reactions across Class I and II SAB lots in unsensitized men

Unsensitized men with Class I Luminex SAB results (n=2649)											
Lot #11 (n=361)			Lot #12 (n=219)			Lot #13 (n=594)			Lot #14 (n=696)			Lot #15 (n=779)		
Allele	Positive (%)	Median MFI (IQR)	Allele	Positive (%)	Median MFI (IQR)	Allele	Positive (%)	Median MFI (IQR)	Allele	Positive (%)	Median MFI (IQR)	Allele	Positive (%)	Median MFI (IQR)
B*15:12	10.8	6090 (3041–9050)	B*57:01	12.79	5575 (3444–8089)	B*15:12	13.13	4002 (2650–9488)	B*15:12	10.34	4623 (2145–8079)	B*15:12	11.55	4667 (2453–13121)
B*57:01	10.53	4133 (2613–8641)	B*57:03	12.33	5318 (3434–14224)	B*57:01	9.93	4170 (2476–15685)	B*45:01	8.62	5713 (3317–11308)	A*25:01	10.4	6119 (2543–14257)
B*57:03	10.53	4323 (2893–9356)	B*15:12	11.87	3771 (2248–7649)	A*23:01	9.76	4203 (2459–11082)	B*57:01	8.19	3522 (2377–9587)	A*66:01	10.27	5576 (3022–14550)
B*58:01	8.86	4009 (2311–8874)	B*58:01	11.42	3485 (2594–7260)	B*57:03	9.26	4268 (2616–15677)	A*24:02	7.33	3931 (2362–8970)	B*57:01	9.76	4696 (2516–9794)
A*66:02	8.59	3040 (2393–9234)	A*24:02	9.59	5716 (2695–15046)	A*24:02	9.09	4148 (2768–10128)	B*44:03	7.33	5154 (2360–13801)	B*45:01	8.86	4487 (2855–16136)
B*44:03	8.59	4973 (2749–13618)	B*44:03	8.68	4400 (2405–10036)	A*25:01	9.09	2961 (2124–13490)	B*57:03	6.61	3886 (2074–9322)	B*57:03	8.86	5291 (2664–9726)
A*02:06	8.31	5302 (3508–17132)	A*23:01	8.22	6718 (2853–14614)	B*45:01	8.42	4353 (2339–10911)	B*82:01	6.61	4825 (2388–10935)	A*24:02	8.73	4989 (2305–14590)
B*44:02	8.31	5621 (2685–14222)	A*25:01	8.22	6620 (2705–13985)	A*24:03	8.25	3543 (2496–9226)	B*08:01	6.47	4375 (2786–9487)	A*24:03	8.6	5561 (2859–16090)
B*45:01	8.31	5378 (2304–12178)	B*44:02	8.22	3623 (2186–8788)	A*66:01	7.74	4233 (2713–13424)	A*02:01	6.32	8572 (3332–18577)	A*23:01	8.34	5977 (2825–14065)
A*02:01	8.03	6373 (4903–17012)	B*45:01	8.22	3373 (2220–7707)	A*34:01	7.41	4710 (2603–8148)	A*24:03	6.32	3862 (2353–7884)	A*26:01	8.34	6375 (3307–14423)

Unsensitized men with Class II Luminex SAB results (n=1355)											
Lot #13 (n=144)			Lot #14 (n=253)			Lot #15 (n=413)			Lot #16 (n=328)			Lot #17 (n=217)		
Allele	Positive (%)	Median MFI (IQR)	Allele	Positive (%)	Median MFI (IQR)	Allele	Positive (%)	Median MFI (IQR)	Allele	Positive (%)	Median MFI (IQR)	Allele	Positive (%)	Median MFI (IQR)
DQB1*03:03 / DQA1*03:02	20.83	15150 (10088–19717)	DQB1*03:02 / DQA1*03:02	20.16	11475 (4895–19903)	DQB1*03:02 / DQA1*03:03	28.75	15079 (13323–18749)	DQB1*03:02 / DQA1*03:03	19.82	10529 (4633–18841)	DQB1*03:02 / DQA1*03:03	21.66	12283 (5589–19948)
DQB1*03:02 / DQA1*03:02	18.75	16440 (11249–20708)	DQB1*03:01 / DQA1*06:01	18.58	11812 (7130–20226)	DQB1*03:01 / DQA1*06:01	20.1	11411 (4997–17165)	DQB1*03:03 / DQA1*03:02	19.21	9211 (4220–19699)	DQB1*03:02 / DQA1*03:01	19.35	12104 (4068–24132)
DQB1*03:02 / DQA1*03:01	18.06	15936 (9349–20957)	DQB1*03:19 / DQA1*05:05	18.58	11755 (6332–21005)	DQB1*03:03 / DQA1*03:02	20.1	11156 (4508–18769)	DQB1*03:19 / DQA1*05:05	19.21	12363 (4620–19134)	DQB1*03:03 / DQA1*02:01	19.35	12860 (4267–20567)
DQB1*03:01 / DQA1*06:01	17.36	14725 (7577–19656)	DQB1*03:01 / DQA1*05:03	18.18	13096 (6839–20870)	DQB1*03:19 / DQA1*05:05	19.85	11649 (5794–17548)	DQB1*03:03 / DQA1*03:01	18.9	9215 (4149–19762)	DQB1*03:03 / DQA1*03:01	19.35	13294 (6568–20815)
DQB1*03:03 / DQA1*02:01	17.36	14788 (6802–20923)	DQB1*03:03 / DQA1*03:02	17.39	11905 (6716–19362)	DQB1*03:02 / DQA1*03:02	19.52	9888 (3411–17950)	DQB1*03:01 / DQA1*05:03	18.6	12252 (4835–20374)	DQB1*03:03 / DQA1*03:02	19.35	15014 (6482–20933)
DQB1*03:19 / DQA1*05:05	17.36	13954 (8360–19918)	DQB1*03:02 / DQA1*03:01	16.6	8448 (4804–19871)	DQB1*03:01 / DQA1*05:03	19.37	11805 (5337–18801)	DQB1*03:01 / DQA1*06:01	17.99	11724 (5165–18755)	DQB1*03:02 / DQA1*02:01	17.97	14655 (7513–21803)
DQB1*02:01 / DQA1*05:01	16.67	13292 (7086–18921)	DQB1*03:01 / DQA1*02:01	16.21	6508 (2643–17085)	DQB1*03:03 / DQA1*02:01	19.13	9457 (3774–19925)	DQB1*03:03 / DQA1*02:01	16.77	10526 (5142–21730)	DQB1*03:01 / DQA1*02:01	17.51	13808 (4485–24751)
DQB1*03:03 / DQA1*03:01	16.67	14213 (7489–19934)	DQB1*03:03 / DQA1*02:01	16.21	6553 (4121–17734)	DQB1*03:03 / DQA1*03:01	18.16	10763 (3647–22072)	DQB1*03:02 / DQA1*03:01	15.85	7727 (4435–19361)	DQB1*03:01 / DQA1*03:01	17.51	14883 (4418–23763)
DQB1*04:01 / DQA1*03:03	16.67	18753 (6891–20573)	DQB1*03:02 / DQA1*02:01	15.81	6226 (4133–16665)	DQB1*03:02 / DQA1*03:01	17.92	11093 (4493–21891)	DQB1*03:02 / DQA1*02:01	15.24	8849 (4347–20667)	DQB1*03:19 / DQA1*05:05	17.05	16635 (7804–19026)
DQB1*03:01 / DQA1*02:01	15.28	12115 (3363–22011)	DQB1*02:01 / DQA1*05:01	14.62	14731 (4370–20786)	DQB1*03:01 / DQA1*03:01	17.68	7751 (2376–25898)	DQB1*02:01 / DQA1*05:01	14.63	16398 (6481–21344)	DQB1*03:01 / DQA1*05:03	16.59	16857 (7947–20016)

CONCLUSIONS

Our findings indicate that spurious SAB reactivity remains detected in unsensitized men with the latest Class I and II SAB lots. As these reactions occurred with moderate to high MFI values, they can compromise the assignment of unacceptable antigens and the accuracy of virtual crossmatches, ultimately disadvantaging these patients on the waiting list. Moreover, since we cannot completely rule out the possibility of misleading data on sensitizing events in all patients, the SAB reactivity in these individuals should be carefully evaluated.

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