CAROLINA KNEIB

AVALIAÇÃO DA RESPOSTA IMUNOLÓGICA HUMORAL ANTI-DOADOR APÓS O IMPLANTE DE ALOENXERTOS VALVARES HUMANOS CRIOPRESERVADOS *VERSUS* DESCELULARIZADOS

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Dedico este trabalho a todos
Que acreditaram em mim,
Em especial ao meu marido Rodrigo
E a minha pequena Mareska.

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“Deus dá a todos uma estrela.
Alguns fazem dela um sol.
Outros nem conseguem vê-la”.
Helena Kolody

RESUMO

Introdução: No transplante valvar com aloenxertos criopreservados, a presença de células que expressam moléculas HLA, como células endoteliais, pode ser responsável pela iniciação da resposta imune contra o aloenxerto valvar. Uma abordagem promissora como alternativa na redução da respota imunológica no transplante valvar é a utilização de valvas cardíacas descelularizadas. **Objetivo:** Comparar o desenvolvimento de anticorpos anti-HLA específicos contra o doador (DSA) em pacientes submetidos ao transplante com aloenxerto criopreservados celularizados versus decelularizados pela solução PUC (SDS – dodecil sulfato de sódio) e determinar os possíveis epitopos imunogênicos responsáveis pela reatividade dos anticorpos. **Métodos:** Amostras de soro dos pacientes submetidos ao transplante valvar com homoenxerto criopreservado (CRIO) ou descelularizado (DESC) foram coletadas no pré-transplante, 5ºPO, 10ºPO, 30ºPO e 90ºPO, e armazenadas à -20ºC até o uso. Os testes foram realizados com o kit comercial Luminex LABScreen® Single Antigen para classe I e II. As amostras que apresentaram anticorpos DSA foram analisadas através do programa HLAMatchmaker, um algoritmo que analisa a compatibilidade receptor-doador através da configuração de aminoácidos, os eplets. **Resultados:** Ao analisar os resultados obtidos, observamos que após o implante valvar, no grupo DESC a resposta imunológica anti-HLA foi menor que no grupo CRIO. Além disso, todos os pacientes do grupo CRIO apresentaram DSA de classe I e/ou II durante o período estudado. No grupo CRIO os possíveis epítopes imunogênicos presentes nas moléculas HLA contra as quais foram formados anticorpos são compartilhados com as moléculas HLA do doador. Já no grupo DESCEL os possíveis epítopos imunogênicos encontrados não foram responsáveis pelo desenvolvimento de resposta imunológica. **Conclusão:** Nosso estudo demonstra que os possíveis epitopos imunogênicos encontrados no grupo celularizado eram anti-doador. Enquanto que no grupo decelularizado não. Sendo assim, sugerimos que a utilização da solução de descelularização PUC (SDS) é uma alternativa efetiva na diminuição da imunogenecidade do transplante de aloenxerto valvar.

Palavras-chave: transplante valvar, decelularização, PRA, HLAMatchmaker.

ABSTRACT

Abstract

We have evaluated the development of humoral antibodies in response to donor allograft valve implant in patients who received cellularized and decellularized allografts and determine possible immunogenic epitopes considered responsible for antibodies reactivity. **Methods:** Sera samples from all recipients who received cellularized allografts (CRYO) or decellularized SDS allografts (DECEL) were collected before valve replacement and at 5, 10, 30 and 90 days post operatively and stored at -20°C until required. Tests were performed using Luminex LABScreen® Single Antigen Class I and II. To determine possible immunogenic epitopes, the samples that presented donor specific antibodies were analyzed using the software HLAMatchmaker. **Results:** We have observed that decellularized grafts elicited lower levels than cellularized allografts regarding levels of class I and class II anti-HLA antibody formation after implantation. Furthermore, all patients from CRYO group presented DSA class I and II within 3 months of observation period. For HLAMatchmaker analysis, in the CRYO group, the result number of immunogenic epitopes were significantly higher than the number for DECEL group, both class I and II (*p*: 0,002 – cl I / *p*: 0,009 – cl II / *p*: 0,004 – cl I and II). **Conclusions:** Our findings can demonstrate that the possible immunogenic epitopes in the cellularized group were against donor HLA molecules. Although in the decellularized group the possible immunogenic epitopes were not against donor HLA molecules. Being so, we suggest that the choice of SDS decellularization process is the best alternative for the decrease of the immunogenicity of allograft valve transplant.

Key words: valve transplant, decellularization process, PRA, HLAMatchmaker.

SUMÁRIO

1 INTRODUÇÃO

O mau funcionamento das valvas pode resultar em sobrecarga do músculo cardíaco, o que torna os pacientes, portadores destas lesões, progressivamente limitados podendo vir a falecer se não tratados cirurgicamente. Em geral, a disfunção valvar pode ser conseqüência de lesões nas valvas cardíacas causadas pela pressão externa em torno do coração, pela presença de deficiência de vitamina B, por patologias primárias do músculo cardíaco ou por qualquer outra anormalidade, como defeitos congênitos, moléstia reumática e degeneração calcifica capaz de transformar o coração em uma bomba hipoefetiva (DOHMEN *et al.,* 2002).

Existem dois procedimentos cirúrgicos que são utilizados para correção do mau funcionamento valvar, a plastia valvar (que consiste em reparar a valva doente) e a substituição ou transplante valvar. A substituição da valva cardíaca por uma prótese valvar é necessária quando a deformidade da valva é tão relevante que só a plastia valvar não resolveria (COSGROVE, 1987). O transplante valvar é um tratamento cirúrgico efetivo das disfunções valvares graves. Para isso é necessário o implante de uma prótese valvar para substituição da valva doente e, portanto, a doença eliminada. As alternativas existentes para o transplante valvar com implante cirúrgico são: o implante de próteses mecânicas (COSGROVE, 1987) e o de próteses biológicas (GOTT *et al.,* 1992).

As próteses mecânicas são feitas a partir de ligas metálicas especiais e têm sido utilizadas com grande freqüência em pacientes jovens (KHAN *et al.,* 2001). A maior vantagem apresentada por estas próteses é a grande durabilidade, porém esta é contrabalanceada pela grande incidência de formação de coágulos sanguíneos, o que ocasiona problemas tromboembolíticos e trombose aguda valvar

(CONCHA *et al.,* 2004). Na trombose aguda valvar, a prótese fica obstruída por coágulos sanguíneos, já os problemas tromboembolíticos são causados por pequenos coágulos formados ao redor da prótese que se desprendem e migram para a circulação periférica, e são embolizados em diversos órgãos, principalmente no cérebro. Sendo assim, após o transplante valvar utilizando próteses mecânicas, os pacientes são submetidos ao tratamento contínuo com anticoagulantes (COSGROVE, 1987).

As próteses biológicas são aquelas produzidas através de tecido humano ou animal. Podem ser divididas em heteroenxertos (tecido animal) e homoenxertos ou aloenxertos (tecido humano), sendo os tipos de heteroenxertos mais comuns o pericárdio bovino e a valva aórtica porcina (HOERSTRUP *et al.,* 2000; GAO *et al.,* 2004). Os homoenxertos ou aloenxertos apresentam funcionamento superior ao de outras próteses, pois apresentam fluxo sanguíneo laminar central e desempenho hemodinâmico fisiológico, evitando gradientes residuais e refluxo valvar. Sendo assim, é quase nula a incidência de problemas tromboembolíticos, excluindo a utilização de anticoagulantes (CHAMBERS *et al.,* 1997). Além disso, o fato dos homoenxertos possuírem hemodinâmica fisiológica satisfatória aumenta a sobrevida em longo prazo nos pacientes transplantados, devido à melhora na regressão da hipertrofia ventricular esquerda (BANDO *et al.,* 1995). Diante destes fatos, acredita-se que os homoenxertos são opções seguras de sobrevida e apresentam desempenho satisfatório no transplante valvar (LEGARRA *et al.,* 2001).

Os homoenxertos podem ser utilizados como próteses biológicas frescas (GAVIN *et al.,* 1973), ou ainda, próteses criopreservadas (ARMIGER *et al*., 1985). As próteses biológicas frescas são homoenxertos provenientes de doador-falecido, captadas sob condições estéreis e mantidas em meio de cultura à 4ºC até o

momento da implantação (GAVIN *et al.,* 1973). Porém, desvantagens como a rápida perda da viabilidade antes da utilização e ainda a presença de degeneração dos folhetos valvares após o implante, comprometem a durabilidade do enxerto e aumentam a necessidade de reoperação (O'BRIEN *et al.,* 1987). A criopreservação trata-se de um processo de preservação e proteção das células e dos tecidos valvares através do congelamento do material escolhido podendo manter-se viáveis por até 9 anos sob correto armazenamento (O´BRIEN *et al.,* 1987).

De acordo em Gerola *et al.* (2004) o resultado imediato do implante de homoenxertos criopreservados apresenta resultados satisfatórios, pois o homoenxerto valvar criopreservado apresentou excelente performance hemodinâmica, com baixo gradiente transvalvar e regurgitação mínima após 30 dias pós-transplante. Por outro lado, Schenke-Layland *et al.* (2006), verificaram que a criopreservação resulta em severas alterações na integridade da matriz extracelular do homoenxerto valvar, ou ainda, destruição das estruturas da matriz extracelular dos folhetos valvares. Além disso, Shapira *et al.* (1995), relataram que pacientes submetidos ao transplante valvar com aloenxerto criopreservado apresentavam febre por motivo desconhecido, ainda no período pós-transplante imediato.

Um dos fatores que pode ter contribuído para a obtenção de resultados contraditórios é o método de preservação dos homoenxertos. Durante os últimos 30 anos, as técnicas de preservação dos aloenxertos valvares sofreram várias mudanças. As técnicas de preservação e manutenção de homoenxertos frescos foram substituídas por técnicas de congelamento. As técnicas de esterilização passaram a ser combinadas com o uso de irradiação, β-propionolactona, clorexidina, formaldeído, e ainda, o armazenamento em solução antibiótica fria (BODNAR *et al.,* 1989). Todos estes aperfeiçoamentos visam à melhoria dos processos que

envolvem a captação da valva, a partir de doador-falecido, além do encaminhamento para o Banco de Homoenxertos, do manuseio, da esterilização e manutenção da viabilidade valvar, bem como do armazenamento das mesmas.

O substituto valvar ideal deve apresentar boa dinâmica de abertura e fechamento completo, ser não obstrutivo, não trombogênico, resistente a infecção, quimicamente inerte, não hemolítico, durável e ser de fácil implantação (HARKEN *et al.,* 1962). Estudos mais atuais têm focado seus esforços nas técnicas de engenharia de tecidos para criar um substituto valvar vivo a fim de solucionar os problemas encontrados no transplante de valvas convencional (FUCHS *et al.,* 2001), como por exemplo, a diminuição ou perda da viabilidade da matriz valvar (ARMIGER, 1995; SCHENKE-LAYLAND *et al*., 2006), a ocorrência de fibrocalcificação (CHRISTENSON *et al*., 2004) e ainda a presença de reação imunológica (FISCHLEIN, *et al*., 1995; BATTEN *et al*., 2001; COSTA *et al.*, 2005).

A escolha da matriz ideal é um ponto crucial de todo o processo, pois deve ser biodegradável e permitir a endotelização e re-população pelas células intersticiais do receptor. Durante o processo, a matriz deve apresentar estabilidade mecânica a fim de suportar o estresse hemodinâmico do fluxo valvar. Além disso, a matriz deve ser biologicamente inerte e ainda não apresentar resposta imunológica e/ou inflamatória específica, ou seja, deve apresentar comportamento semelhante ao de uma valva humana cardíaca nativa (MAYER *et al.,* 1997; HOERSTRUP *et al*., 2000; KASIMIR *et al.,* 2005).

É conhecido que a maior fonte de antigenicidade dos homoenxertos valvares está nos elementos celulares, como fibroblastos e células endoteliais, pois resultam na ativação de linfócitos T e produção de anticorpos contra os antígenos HLA de classe I e II (SCHLITZ *et al*., 1994; SMITH *et al*., 1995).

Hawkins *et al.* (2000) ao estudar a presença dos anticorpos anti-HLA de classe I e II após o implante de aloenxertos criopreservados em crianças, concluíram que em 3 meses de observação, os aloenxertos criopreservados induziram uma resposta imunológica contra os antígenos HLA de classe I e II , e que esta resposta pode representar uma forma de rejeição, levando a complicações para aqueles onde o transplante valvar é um paliativo para o transplante cardíaco ou ainda, a uma falha precoce do aloenxerto.

Da mesma forma, Welters *et al.* (2002) observaram que 89% dos pacientes submetidos ao transplante com homoenxertos criopreservados apresentaram anticorpos anti-HLA, sendo que o título dos anticorpos anticorpos anti-HLA específicos contra o doador apresentaram um aumento significativo em pacientes submetidos a um retransplante .

Além disso, Smith *et al.* (1995) estudaram a resposta humoral contra homoenxertos valvares aórticos através da detecção de anticorpos anti-HLA pelo método de linfocitotoxicidade. Neste estudo eles observaram que a presença de anticorpos anti-HLA foi maior nos pacientes submetidos ao transplante valvar com homoenxerto fresco provenientes de receptores de transplante de coração ou pulmão-coração do que naqueles que foram transplantados com homoenxertos esterilizados provenientes de doadores cadáveres. Sugerindo que o tipo de tratamento do aloenxerto pode ter influência na redução da imunogenicidade do aloenxerto.

A possibilidade de reação imunológica proveniente dos componentes celulares das próteses de valvas cardíacas humanas torna essencial a eliminação parcial ou total destes componentes para obtenção de uma matriz natural apropriada e conseqüente pós-transplante satisfatório. É o que demonstraram Cebotari *et al*.

(2002) e Booth *et al*. (2003), quando descrevem a utilização de homoenxertos descelularizados como uma nova alternativa para o implante de homoenxertos valvares.

Os homoenxertos descelularizados são aqueles submetidos às técnicas de engenharia de tecidos que visam eliminar as células do homoenxerto, mantendo a matriz extracelular preservada. Assim, o homoenxerto descelulariazado é implantado no paciente esperando-se que na ausência das células do enxerto, não ocorra resposta imunológica (DOHMEN *et al.,* 2002; O´BRIEN *et al.,* 2001). Vários processos químicos capazes de descelularizar as valvas cardíacas têm sido propostos, como o tratamento das valvas criopreservadas com tripsina (STEINHOFF *et al.,* 2000), dodecilsulfato de sódio (SDS) (BOOTH *et al.*, 2002), octilphenoxietoxietanol (Triton X-100) (BADER *et al.*, 1998), ácido deoxicólico (DOMEHN *et al.*, 2002), enzimas como RNAse e DNAse (GRAUSS *et al.*, 2003), etanol e glicerol (WILSON *et al.*, 1995).

Cebotari *et al.* (2002) utilizaram tripsina/EDTA para descelularizar homoenxertos valvares *in vitro*, resultando em uma matriz extracelular preservada, a qual foi posteriormente rescelularizada com células endoteliais humanas, resultando em uma prótese modificada com metabolismo e durabilidade satisfatórios. Elkins *et al.* (2001), Bechtel *et al. (*2003) e Costa *et al.* (2005) estudaram a resposta humoral em pacientes transplantados com valvas cardíacas descelularizadas através dos métodos SynerGraft™ e Auto Tissue Ltd™. e observaram que este processo auxiliou na redução da resposta imunológica, mantendo o enxerto com função satisfatória.

Entretanto, o objetivo de diminuir ou eliminar a imunogenicidade dos homoenxertos através das técnicas de descelularização algumas vezes pode não ser alcançado efetivamente (SHINOKA, 1998). Desta forma, o estudo do impacto da

formação de anticorpos específicos contra o doador (DSA - *donor specific antibodies*) na funcionalidade do enxerto torna-se fundamental para a avaliação dos métodos de descelularização.

Avanços na tecnologia de purificação de antígenos HLA levaram ao desenvolvimento de metodologias alternativas para estes painéis, tais como ELISA (*Enzime Linked Immuno Sorbent Assay*) e CFFS (Citometria de Fluxo em Fase Sólida). Estes testes detectam anticorpos anti-HLA com maior sensibilidade e permitem a definição das suas especificidades anti-classe I e II (ZEEVI e GIRNITA e DUQUESNOY, 2006). O teste mais sensível disponível hoje é o CFFS com micro-esferas ligadas a um único antígeno realizado na plataforma multiplex. Este teste é considerado 25 % mais sensível do que o ELISA (HERCZYK, 2003).

Aliado a caracterização dos anticorpos anti-HLA, a utilização do programa HLAMatchmaker possibilita a determinação dos possíveis epítopos responsáveis pela resposta imunológica contra o aloenxerto (DUQUESNOY, 2006). Este programa considera duas características distintas dos epítopos, denominadas antigenicidade (a reatividade ao anticorpo) e imunogenicidade (habilidade de induzir resposta ao anticorpo). A imunogenicidade depende da diferença estrutural entre um antígeno imunizado e uma molécula HLA que responde ao anticorpo. Algumas diferenças estruturais levam a epítopos imunodominantes visto que outros estão associados com baixa imunogenicidade. A antigenicidade reflete as exigências estruturais para um epítopo reagir a um anticorpo específico, sendo que a variabilidade seqüencial, estrutura secundária e influências conformacionais de resíduos próximos podem afetar a antigenicidade do epítopo. (DUQUESNOY, 2009).

Desta forma, o desenvolvimento de técnicas de descelularização eficientes aliadas ao estudo detalhado dos anticorpos anti-HLA presentes no soro do paciente

pode tornar a sobrevida do transplante de valvas cardíacas com homoenxertos cada vez mais satisfatória e duradoura.

2 OBJETIVO

Este trabalho teve como objetivos:

- ✓ Comparar o desenvolvimento de anticorpos anti-HLA específicos contra o doador em pacientes submetidos ao transplante com aloenxertos criopreservados celularizados *versus* descelularizados com a solução PUC;
- ✓ Comparar os possíveis epítopes imunogênicos presentes nas moléculas HLA contra as quais foram formados anticorpos durante o monitoramento pós-transplante com as moléculas HLA do doador, utilizando o programa HLAMatchmaker.

3 ARTIGO

EVALUATION OF HUMORAL IMMUNE RESPONSE TO DONOR HLA AFTER IMPLANTATION OF CRYOPRESERVED VERSUS DECELLULARIZED HUMAN HEART VALVE ALLOGRAFTS

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Abstract

We have evaluated the development of humoral antibodies in response to donor allograft valve implant in patients who received cellularized and decellularized allografts and determine possible immunogenic epitopes considered responsible for antibodies reactivity. **Methods:** Sera samples from all recipients who received cellularized allografts (CRYO) or decellularized SDS allografts (DECEL) were collected before valve replacement and at 5, 10, 30 and 90 days post operatively and stored at -20°C until required. Tests were performed using Luminex LABScreen® Single Antigen Class I and II. To determine possible immunogenic epitopes, the samples that presented donor specific antibodies were analyzed using the software HLAMatchmaker. **Results:** We have observed that decellularized grafts elicited lower levels than cellularized allografts regarding levels of class I and class II anti-HLA antibody formation after implantation. Furthermore, all patients from CRYO group presented DSA class I and II within 3 months of observation period. For HLAMatchmaker analysis, in the CRYO group, the result number of immunogenic epitopes were significantly higher than the number for DECEL group, both class I and II (*p*: 0,002 – cl I / *p*: 0,009 – cl II / *p*: 0,004 – cl I and II). **Conclusions:** Our findings can demonstrate that the possible immunogenic epitopes in the cellularized group were against donor HLA molecules. Although in the decellularized group the possible immunogenic epitopes were not against donor HLA molecules. Being so, we suggest that the choice of SDS decellularization process is the best alternative for the decrease of the immunogenicity of allograft valve transplant.

Key words: valve transplant, decellularization process, PRA, HLAMatchmaker.

INTRODUCTION

Valve transplant with biological tissue have been used since 1962 (1). Allograft valves are the most useful biological prostheses for valve replacement (2-4).Although these prostheses are efficient and reduce substantially the morbidity and mortality. However there still a design issue and a non effective response of the body to the implanted materials.(5). In aortic position, allograft present a significant structural deterioration in the first 10 years post implantation (6-7), and in pulmonary position, graft stenosis can prevent long-term durability (8). Most of patients who received cryopreserved allograft developed humoral antibodies against human leukocyte antigens (HLA), which are specific against transplanted tissues (9-11).

In organ transplantation, the presence of donor-derived dendritic cells in allogenic tissue has been suggested to play an important role in the immune activation of recipient, by direct antigen preservation (12). Studies have shown that the absence of dendritic cells (antigen presenting cells) in cryopreserved valve allografts is compensated by the preservation of other cells expressing HLA class II molecules predominantly in the endothelium which may be responsible for initiation of a specific immune response against heart valve allograft (13).

Tissue engineering has been used to overcome these limitations. A promising approach using decellularized heart valve allografts (14), make us believe and wait for avoiding the immune response (4). Experimental and clinical experiences with decellularization process has been gained with porcine tissue (15), ovine tissue (16) and subsequently, human tissue (4). Several methods have been developed to produced completely acellular heart valve tissue matrices using multistep detergent-enzymatic extraction (17), Triton detergent (15), trypsin/ethylenediaminetetraacetic

acid (18), deoxicolic acid (19), RNAse and DNAse (14). Meyer (2005) studied in rats, the reduction of immune response to aortic valve allografts by the decellularization process. They concluded that decellularization significantly reduces the cellular and humoral immune response to allograft tissue.

Zehr (2005) and Bechtel *et al.* (2003), provides convincing evidence that the SynerGraft™ decellularization technology successfully removed antigens from an aortic and pulmonary allograft. Another protocol, using sodium dodecyl sulfate (SDS) in the presence of protease inhibitors, was successful for heart valve decellularization (23). Costa *et al*. (2005), demonstrated that decellularized allografts are less immunogenic that cryopreserved allografts and had normal and stable hemodynamic performance up to 18 months post-operatively. They compared the immunological and echocardiographic data of decellularized (AutoTissue Ltd™) versus cryopreserved allografts used for right ventricular outflow tract (RVOT) reconstruction during Ross operation. By the way, the use of decellularized allograft has been chosen as an alternative for post transplant immunological responses in the cryopreserved valve implant.

To evaluate the efficacy of the decellularization process for decreasing or preventing the development of humoral antibodies in response to donor allograft valve implant, the presence of anti-HLA antibodies was evaluated using the panel reactive antibodies (PRA) by flow citometry technology in patients implanted with a cryopreserved allograft valve (CAV) or AutoTissue Ltd™-treated allograft valve (ATAV) or SDS-treated allograft valve (SDSAV). Additionally, the HLA class I and II mismatches between recipient and donor were analyzed with the HLAMatchmaker program (25), to determine the possible immunogenic epitopes that were responsible for the antibodies reactivity.

MATERIALS AND METHODS

Patients

Between 2005 October and 2009 March, twelve patients underwent an aortic or pulmonary valve replacements which were studied prospectively. A Group 1 (CRYO) consisted of six patients who received cryopreserved allograft valve (median age 59 years, age range 30-75 years; 04 male, 02 female, 05 aortic valve and 01 pulmonary valve) and a Group 2 (DECEL) with 6 patients who received sodium dodecyl sulfate (SDS) 0,1% decellularized allograft valve (median age 38.6 years, age range 24-49 years; 01 male, 05 female, 01 aortic valve and 05 pulmonary valve). The preoperative and surgical characteristics of the patients are listed in Table 1 and 2. Despite the group heterogenicity show no major differences which influence in the results were found between the two groups. The choice of allograft size was done according to patient surface area, but as general rule we tried to implant the biggest allograft available with a deliberate over sizing policy. The study was conducted in accordance with institutional guidelines and has been approved by the Ethical Committee of Pontificia Catholic University of Parana (PUCPR) with the protocol number 1305. Before being enrolled, patients signed the informed consent to participate in the study.

Table 1. Group 1 – Cryopreserved Patient information

Patient	*Gender*	*Age (years)*	*Valve Origin*	*Indication*	*Valve Size*	*Hemoderivative*	*ABO*
PAC1C	M	56	AORT.	AORTIC STENOSIS	26	ERYTHROCYTES	O-
PAC2C	F	51	AORT.	AORTIC INSUFF.	23	PLATELETS, PLASMA, ERYTHROCYTES	A+
PAC3C	M	69	AORT.	AORT. ANEURYSM	22	PLASMA, ERYTHROCYTES	A+
PAC4C	F	75	AORT.	AORTIC STENOSIS	22	PLATELETS, PLASMA, ERYTHROCYTES	NI
PAC5C	M	73	AORT.	DOUBLE AORTIC LESION	22	PLATELETS, PLASMA, ERYTHROCYTES	NI
PAC6C	M	30	PULM.	AÓRTIC INSUFF.	24	ERYTHROCYTES	O-

NI: not informed

Table 2. Group 2 – Decellularized patient information

Patient	*Gender*	*Age (years)*	*Valve Origin*	*Indication*	*Valve Size*	*Hemoderivative*	*ABO*
PAC1D	F	48	PULM.	AORTIC STENOSIS	23	PLASMA, ERYTHROCYTES	O+
PAC2D	F	23	PULM.	AORTIC STENOSIS	24	ERYTHROCYTES	O+
PAC3D	F	44	PULM.	AÓRTIC INSUFF.	23	PLASMA, ERYTHROCYTES	O+
PAC4D	M	25	PULM.	AORTIC STENOSIS	24	PLATELETS, PLASMA, ERYTHROCYTES	NI
PAC5D	F	49	PULM.	AÓRTIC INSUFF.	24	PLATELETS, PLASMA, ERYTHROCYTES	O+
PAC6D	F	43	AORT.	AÓRTIC INSUFF.	23	ERYTHROCYTES	B+

NI: not informed

Operative Technique

All operations were done through a median sternotomy with cardiopulmonary bypass and mild to moderate systemic hypothermia (30-32ºC). Myocardial protection was achieved with administration of doses of intermittent antegrade cold blood cardioplegia through the coronary ostia every 10-30 min. The pulmonary autografts were implanted as a root replacement in all cases and the RVOT was reconstructed with interposition of an allograft with running sutures of polypropylene 4-0 for both the proximal and distal sutures lines. There was no extension of the allograft with pericardial patches in the proximal suture line in any case.

Allografts Preparation

The allografts were obtained from heart beating donors and all the treatment of theses allografts were prepared by the Human Cardiac Valve Bank of Santa Casa of Misericordia of Curitiba (BVCHSC). The cryopreservation was done according to previously published methods (26). The valves were prepared according standard protocols and decellularized by a proprietary process (27). All allografts were first cryopreserved, and when required were decellularized. The choice of the valve prostheses occurred according to the patient needs, allograft availability and implantation technique.

HLA typing of valve donor and recipient

Blood samples with EDTA anticoagulant were collected from all valve donors and obtained from allograft valve bank. Recipients' blood samples with EDTA anticoagulant were obtained before or after valve transplant. All recipients and valve donors were typed for HLA-A, -B and –DR (PCR-SSO LABType® Class I and II - One Lambda Inc.).

Anti-HLA antibodies detection

Sera samples from all recipients were collected before valve replacement and at 5, 10, 30 and 90 days postoperatively and stored at -20ºC until required. The tests were done using the panel reactive antibodies (PRA) by Flow Citometry (LABScreen® Single Antigen Class I and II). These methodology uses microbeads coated with purified Class I or Class II HLA antigens and pre-optimized reagents for the detection of class I or class II HLA antibodies in human sera. In Single Antigen, singles beads are used to focus on reactions against one or a few antigens, e.g. to compare reactivity of different serum samples from the same individual (28). The data acquisition was done by the LABScan™ 100 flow analyzer (Luminex) that detects the fluorescent emission of each bead. The obtained results are then analyzed at the HLA Fusion® software. The cut off used was the same used at our lab protocol, which determines for the cut off value any MFI (medium of fluorescent intensity) value over 500.

HLAMatchmaker

Principle of HLAMatchmaker

HLAMatchmaker is a computer program that determines the HLA compatibility at the structural level (25,29). In the HLAMatchmaker each HLA antigen is viewed as a string of epitopes represented by short sequences involving polymorphic amino acid residues in antibody-accessible positions and by longer sequences and amino acid residues in discontinuous sequence positions which are called eplets. These eplets are considered key elements of epitopes that can elicit specific alloantibodies (29).

Analysis of Antibody Reactivity Patterns with HLAMatchmaker

The positive PRA results of this study were included at algorithm program named HLAMatchmaker (2008 version) that determines HLA compatibility at the structural level (29). The first step of a HLAMm serum analysis is to identify those alleles that are responsible to reveal negative antibodies reactions. Such alleles can be expected to have eplets that are not recognized by patient's antibodies and from theses eplets together with the patient's own eplets we can identify the eplets that may be responsible for the positive antibodies reactions, and furthermore, if the possible immunogenic eplets are from donor specific antibodies.

Statistical analysis

The obtained results were expressed by median, minimum values and maximum values, or by frequencies and percentage. For group comparison regarding antigenic epitopes number was considered non-parametric test of Mann-Whitney. For the probability of antigenic epitopes presence, the groups were compared using the Fisher test. $P<0,05$ was statistical significant. The data were organized in a excel base and analized with the computer program Statistica 8.0.

RESULTS

The initial analysis was done observing two groups, the HLA compatibility between recipients and donors. We observed that for all recipients the incompatibility was higher than 3 mismatches. The next step was the Single Antigen PRA analysis. We have demonstrated that decellularized grafts elicited lower levels than cellularized allografts regarding levels of class I and class II anti-HLA antibody formation after implantation. For CRYO group we observed that two recipients presented class I DSA (donor specific antibodies) in the pretransplant following all the studied period, and developed class II DSA during the post transplant monitoring. The others four recipients of the same group developed class I and II DSA after the transplant (Table 1). In the DECEL group, one recipient did not present anti-HLA antibodies in any analyzed sample. Two presented class I DSA already in the pretransplant samples and class II DSA within 3 months of the observation period. The others three patients developed class I DSA during the post transplant monitoring, but for class II, one did not develop anti-HLA antibodies, other presented class II DSA in the pretransplant sample, and the last one developed class II DSA during the observation period (Table 4). After statistical analysis, we observed that the development of DSA in posttransplant of the two groups (CRYO and DECEL) was statistically significant only for class II (p=0,015). All patients received a type of hemoderivatives which included platelets concentrate, fresh plasma and/or erythrocytes concentrate (Table 1 and 2). The type of hemoderivates received did not make difference in the analysis.

Table 4 – Analysis of DSA presence in decellularized and cryopreserved allograft groups.

	DECELLULARIZED ALLOGRAFT		CRYOPRESERVED ALLOGRAFT		
	CLASS I	CLASS II	CLASS I	CLASS II	
PAC1D	**B**	**D**	**D**	**D**	PAC1C
PAC2D	**A**	**A**	**D**	**D**	PAC2C
PAC3D	**B**	**C**	**B**	**D**	PAC3C
PAC4D	**D**	**C**	**B**	**D**	PAC4C
PAC5D	**C**	**A**	**D**	**D**	PAC5C
PAC6D	**D**	**B**	**D**	**D**	PAC6C

A	**PRA NEGATIVE**
B	**DSA in the pretransplant sample**
C	**Presence of NO donor specific immunogenic epitopes**
D	**Presence of donor specific immunogenic epitopes**

The patients that presented DSA in the pretransplant sample could not be considered developed against the allograft, because already existed before the transplant. But there was a tendency for higher levels of these antibodies in the post transplant monitoring, for both groups. When we analyzed the PRA results at the HLAMatchmaker program, we observed that the antibodies detected in post transplant serum of decellularized patients did not share or share only few eplets with donor-specific antibodies in comparison of cryopreserved graft group (Table 5). We could observe at CRIO group that almost all antibodies reactions shared possible immunogenic eplets with donors HLA molecules (Table 5).

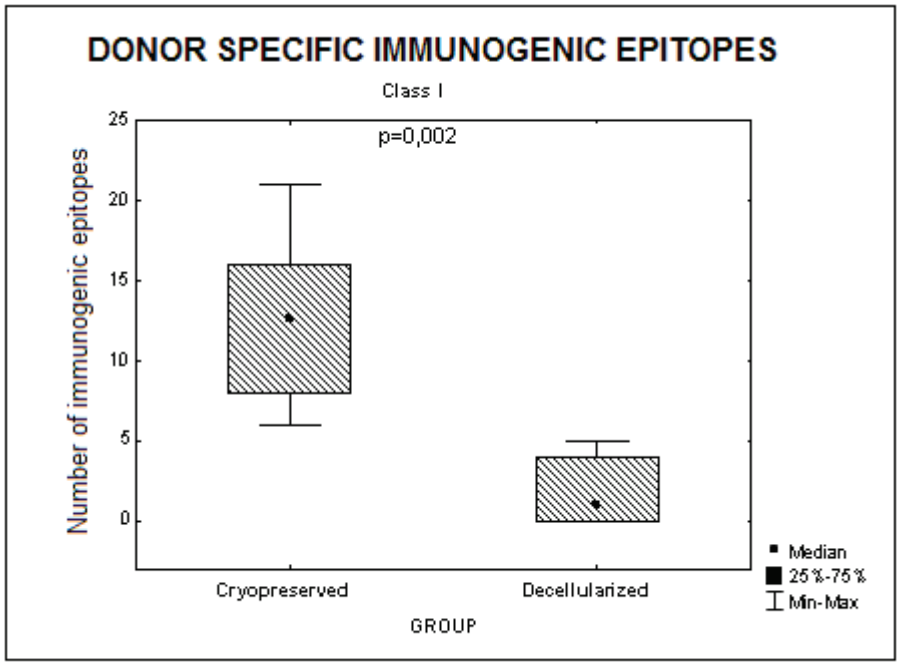
Table 5 - Immunogenic eplets for specific donor molecules defined by HLAMatchmaker analysis. The underlined and in bold eplets represent the possible immunogenic eplets that are shared with the HLA molecules that presented positive anti-HLA antibodies reactions after cardiac valve transplant.

GROUP	Patients	IMMUNOGENIC EPLETS FOR SPECIFIC DONOR MOLECULES
GROUP 1	PAC1C	,,,,,45RMA,62GE,,,,,71SA,,76ENI,,79RI,,,,,,,113HD,116S,,,,,,163LW,,,,,,,,, 4Q,,,,,,26QF3,,,,,,,,,,,67IR,,,,,76GDT,180VM,,,98ES,,,,, 4Q,,,,,,,,,32IYN,,,135S,48YQ6,,,,67LR,70LRRA,71RRA,73AEDT,74RRAE,,,,,98KN,,120N,,, ,,,,,,,,,,,,,,,,,,,,,,,,135G,,, ,,,,,45GV,,,,,,,,,,,,,,,,,,,,,,185I ,,25FT,34HE,,,47EK2,48LF,,,,,,75ILR,80IRS2,,,,,,,
	PAC2C	9T,56R,,,,,,,,,,,73ID,76VDT,,,80VGT,,,,,,113YQ,,,,,,,166TEW,,,193AV,245AS,253Q,,,, 9T,,,,,,,,,,,,73ID,76VDT,,,80VGT,,,,,,113YQ,,,,,,,166TEW,,,193AV,245AS,253Q,,,, ,,,,,,,,,,,,,,,,,,,,,,116F,,,,,158T,166TEW,,,,,,,,, ,,,14GEY,,25YRF,,,,,,,47DYR,,57SA,,,,,,73ALDT,,,,,,,,,,, ,142M3,,,,,,26KFD,,,,,,,,,,,71QAA,,,,,,96QV,,,,,, ,,,,,,,,31QDIY,,,40HFD,,,,,,,,,,,,,96EV,,108T3,,,, ,,,26G,,,,,,,57LD,,66DI,,70ED,,74SV,,,,,,,,,,,, ,,,,,,,52PQ,,56RPD,,,,,70GT,71VGT,,,84EV,85EVAG,87AF,90AGI,,125GQ,,,140A2,, ,,,,,44TA2,47QRW,50EF8,,,,,75IMR,,,,,,, ,,,,,,,,,,,69T,,,,,,,,
	PAC3C	9F,,,,,,62GE,,66RKH,,,,73TD,76VDT,,79GT,80VGT,,,,107W,,,,142MT,145KHA,,,,166TEW, ,,,,,,,,66RNQ,,,71QS,73TD,76VDT,,79GT,80VGT,,,,,,113YR,,142MT,145KHA,,,,166TEW,,,,245VA, 9H ,,,,,,,,,,,,,,,,,,,,,113HN,,143ISQ,147L,,,,,163EW,177DT,180E,,,,,,, ,,,,44RT,,,,,,,,,,,,,,,,,,113HN,,,,,,,,,,193PV,,,,,, ,,,,,,,26KFD,,,,,,,,,,,71Qk/rA,71QKA,,,,,,96YL4,,,120N,,, ,12RKS,,,,25HRY,26RY,26KYD,,,,,,,,,,,,,73GRDN,,,,,,,,,,, 4Q,,,,,,,,,32IYN,33LYNQ,,135S,48YQ6,,,,,,,,,,,,98KN,,120N,,, ,14AM,,26Y,30YYA,45EV,,52PL,55PPP,56PPD,57PD,,,67VVT,70RT,71VRT,74EL,77DT,84QL2,,,,116V,,,,140T2,167HG, ,14GM,,26L,30HYA,,,,,56RPD,57PD,,,67VVT,70GT,71VGT,74EL,77DT,,,87AF,,116V,125GQ ,,,,,,,48LF,56RR5,60QF,64TI4,69L,,80IRS2,,,,,160DD,,
	PAC4C	,,,,,,62GE,,66RKH,,70KAH,,,76VDT,,79GT,80VGT,,127K,105S,107W,,,116Y,142MT,145KHA,150AAH,151AHV,,,,,,,,,,, 9T,56R,,,,,,,,,,,73ID,76VDT,,79GT,80VGT,,,105S,,,,,,,,,,,,,,,,,,, ,,,,,,,,,,70IAQ,,,,,,,,,,,,,116Y,,,,,152RE,,177DK,180E,,,,,,, ,12AMR,,41T,44RK,,,,,,,,,,,,,,,,103L,,113YN,116L,,,,,152RE,163LW,,,,,,,,, ,12STS,,14SEH,,,,26TFD,31YYFY,,,,,,57DE,,,,,,,,,,,96HV,,,,140T2,149H, ,12STS,,14SEH,,,,26TFD,31YYFH,32FHN,33LHNQ,,,,,,,,,71DEA,,74DEAA,,,,96HV,,,,140T2,149H,
	PAC5C	9F,,,,,,62GE,,66RKH,69RAHT,70KAH,71HS,,,,,,,127K,,107W,,113YH,,142MT,145KHA,150AAH,151AHV,,,,,,,,,,, 9Y,,,,,,,,,,,,,,,,,,,90D,,,,,,,144KR,150AAH,151AHA,152HA,163RW,,,,,,,,, ,12STS,,14SEH,,,,26TFD,31YYFY,,,,,47DFR,48FR,57DE,,,67FR,70FDRA,71DRA,73AADT,74DRAA,,,,,98KS,,,,149H, ,12STS,,14SEH,,,,26TFD,31YYFH,32FHN,33LHNQ,,,,,57AA,,,,,,,,,112Y,,,98KS,,,, 149H, ,12LKS,,14SEH,,,,,,,32FHN,33LHNQ,,,,,,,67LK,,71QKG,,74QKGQ,76GDN,51R,,,98QS,,,,, ,12LKS,,14SEH,,,,,,,32FHN,33LHNQ,,,,,,,67LK,,71QKG,,74QKGQ,76GDN,51R,,,98QS,,,,, ,14GL,,26G,30HYV,,,52PQ,,,57PV,,,,,70GA,,74SV,,84EV,85EVAG,87AY,90AGI,116I,125SQ,,,,, ,,,,,,,52PQ,,56RPD,57PD,,,,,70GT,71VGT,,,84EV,85EVAG,87AF,90AGI,,125GQ,,,
	PAC6C	,,,,,,,,66RNQ,,,71QS,,,,,,,,,,,,,,,,151AHE,161D,,,,,,,,,, 9Y,,,,44RE,,62RN,65QIA,,,70IAQ,,,,,,,,,,,,,,,,,,152RE,,177DK,,,,,,,, ,,,14FEH,,25HRY,26RY,26KYH,31QGIY,,,40HFD,,,57VA,,,,,,,,,180VM,,,98ES,,,,, ,14GM,,26L,,45GV,,,,,56PPA,57PA,,,,,,,,,,,,,,,,,167RG,185I ,14GM,,26G,,45GV,,,,,57LD,,66DI,,70ED,,74SV,,,,,,,,,,,167RG,185I
GROUP 2	PAC1D	30YYA,,,52PL,55PPP,56PPA,,,,67VVT,70RT,71VRT,74EL,77DT,,,,,,,,,140T2,,185I ,,34HE,,,,48LF,56RR5,,,,,80IRS2,,,,,,
	PAC2D	NO IMMUNOGENIC EPLETS
	PAC3D	NO IMMUNOGENIC EPLETS
	PAC4D	,,,,,,62GE,,66RKH,,70KAH,,73TD,76VDT,,,80VGT,,127K,105S,107W,,113YH,,142MT,145KHT,,,, 166TEW,,,,,
	PAC5D	NO IMMUNOGENIC EPLETS
	PAC6D	9S,,,,,,62EE,,66GKH,,70KAH,,,,,,,,,127K,,,,113YH,,,144KR,150AAH,151AHV,,163TG,167DG,,,,,,,, ,,,,16HFR,25HRF,,26KFD,,,,,,,,,,,,71Qk/rA,71QRA,73AADT,74QRAA,,,,96YL4,,,,, ,142M3,,,16HFR,25HRF,,26KFD,,,,,,47DFR,48FR,,,,,,,71QAA,73AADT,,,,,,96Q ,,,,16HFR,25HRF,,,31QDIY,,,40HFD,,,,,,,,,,73AADT,74DRAA,,,,96EV,,108T3,,

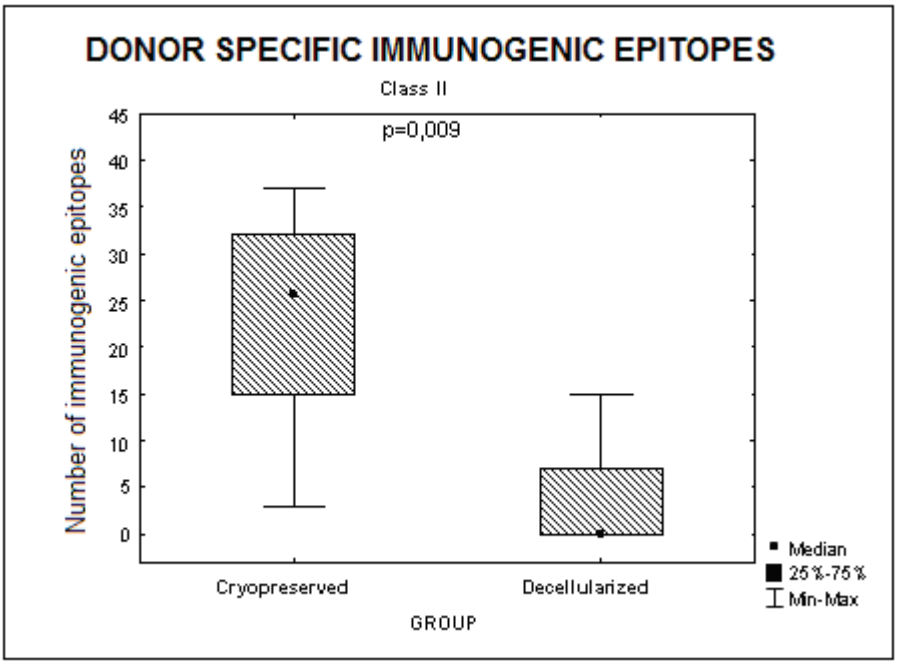
Additionally, we observed that the number of donor specific immunogenic eplets was significantly higher in group 1 (CRYO) than group 2 (DECEL) (graphic 1, 2 and 3). In group 1 (CRYO) we found a median of 12.5 for class I, 25.5 for class II and 37 for class I/II. While, in group 2 (DECEL) we have found a median of 1 for

class I, 0 for class II and 3 for class I/II, where p was significant for all analysis p=0,002 classI, p=0,009 class II and p=0,004 class I/II).

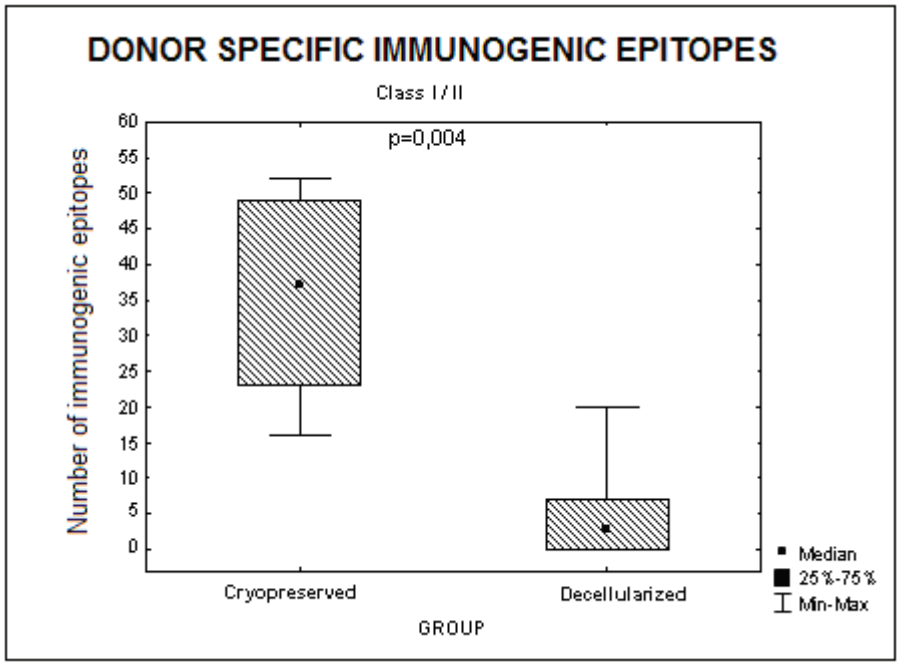
Graphic 1 – Comparison of Donor Specific Immunogenic Epitopes for Class I



Graphic 2 – Comparison of Donor Specific Immunogenic Epitopes for Class II



Graphic 3 – Comparison of Donor Specific Immunogenic Epitopes for Class I/II



DISCUSSION

One of the most significant finding of Single Antigens PRA test is to determine the molecular specificity of alloantibodies (28). The information of donor HLA typing in addition of alloantibodies identification can help us to characterize the possibility by donor specific antibodies. All the patients of the study were analyzed by this method, matching donor HLA typing with patient pre and posttransplant PRA Single Antigens results. After this analysis, we have demonstrated that antibodies reactions in the posttransplant of cryopreserved allografts transplant are directly related to donor specific immune response, what have not show in the decellularized allografts group. These findings can lead us for evidences of reduced immune response following decellularized valve transplant and the use of cryopreserved allografts could induce a higher donor specific immune response.

Even short posttransplant monitoring period (90 days post operatory) was possible to verify an outstanding presence of anti-HLA antibodies and not only donor specific antibodies, in the cryopreserved group, and in many cases an increase in the mean value of fluorescence intensity (MFI) of these alloantibodies during the sera monitoring. However, the MFI of antibodies in decellularized patients group did not present this conduct, what can corroborate with Fischlein *et al*. (1995) when reported that cryopreservation of allograft valve represents a cell- and tissue-protective preservation. However, this authors also have showed that all allograft valves caused immunologic reactions postoperatively, probably because of graft endothelium cell membranes are human lymphocyte antigen class I and II positive and endothelial antigens present the primary immunologic stimulus.

Moreover, Welters *et al*. (2002) demonstrated that cryopreservation allows

preservation of endothelial and valve architecture and viability of presumed improved durability and convenience of long-term storage. Unfortunately, better preservation of allograft and endothelium viability may actually sustain immunogenicity and elicit a more vigorous immunologic reaction from the recipient; this response can theoretically contribute to accelerated degeneration of allograft valves or patch material. In other hand, Yap *et al*. (2006) studied the influence of anti-HLA antibodies in cryopreserved allograft valve implantation and concluded that the clinical significance of their findings is unclear, as no correlation was found between the prevalence of anti-HLA antibody and echocardiographic parameters of valve dysfunction at a mean of 3.5 years follow up.

When we studied the alloantibodies development for the choice of the best process used in valve allografts, we observed that the two different protocols influenced by different ways in the pre and posttransplant of valve allografts patients. We could see that in a first moment (between 3 months) decellularization process present more benefits for valve allograft transplant than only cryopreservation process. This find corroborate with Elkins et al. (2001) when demonstrated that decellularization process is a great method for attempt to reduce the antigenic response in cryopreserved tissue.

The requirements for tissue-engineered heart valves include several potential advantages over currently used prostheses. These goals are: a potential growth capacity, a greater durability and the opportunity to use viable, autologous cells that can utilize the body's mechanisms for repair and remodeling (15). Several distinct methods of decellularization have been employed, and it can be explained for the disparities in the experimental and clinical outcomes (16). Meyer *et al.* (2005) demonstrated that decellularization of aortic valve allografts is associated with a

significant reduction in cellular and humoral immune responses to levels show with nonimmunogenic syngenic tissue. They thought that this could prolong the durability of valve allografts and might prevent immunologic sensitization of allografts recipients. In the same way, Costa *et al.* (2005) evaluated the ELISA PRA results and echocardiographic exams. They observed that decellularized allografts (AutoTissue Ltd^{TM}) were less antigenic than cryopreserved allografts, exhibited normal hemodynamic performance in the right side of the circulation and have yielded stable results up to months postoperatively.

Dignan *et al*. (2003) have shown that HLA class II antigens mismatch was significantly associated with structural degeneration in patients receiving an aortic allograft valve who were followed for five or more years. They also demonstrated a trend toward increased structural deterioration in patients with two or more mismatches of class I, B antigens. Likewise, in our findings, the HLA antigens mismatchs were higher than 3 mismatches, and they appeared more for HLA class II.

In addition to the analysis of presence of donor specific antibodies, we also analyzed the presence of possible immunogenic epitopes specifics to donor HLA molecules. This analysis was done by the HLAMatchmaker program which can be used not only for HLA compatibility studies also to analyze serum screenings for sensitized patients (30).

One of the concepts of HLAMatchmaker is that HLA typing differences between antibody producer and immunizer will define the mismatched eplet repertoire which the patient has been exposed and this information facilitates the interpretation of serum screening results (29). Analysis of the antibody reactivity patterns with HLA panels may distinguish reactive and nonreactive eplets so that specific donor HLA response can be confirmed and the responsible eplet for the

response might be determined.

Our data showed that the immune response, especially in the cryopreserved group, was managed by donor specific epitopes. These findings were possible because we had the HLA typing information of the patients and their valve allograft donor. When we match the possible immunogenic eplets present in the serum screening of the patients with donor HLA typing, we observed that the majority of these eplets were shared with donor HLA molecules, and this observation was significant in cryopreserved group. Cai and Terasaki (2008) reported that the identification of the HLA epitopes should be helpful distinguishing donor specific antibodies from natural antibodies, which appear to be produced in response to non-HLA environmental stimuli.

This kind of analysis is pioneer in valve allograft transplant, but we can find data about another kinds of transplant as heart transplant and most commonly renal transplant. Kosmoliaptsis *et al.* (2008) demonstrated that the number of epitopes mismatched between an alloantigen and the host HLA type determined using the HLAMatchmaker algorithm, correlates closely with both development and strength of an alloantibody response. Dankers *et al.* (2004), a further analysis of sera from patients who had rejected kidney allografts indicated that the chance for a patient develop donor specific alloantibodies directed against mismatched HLA class I antigen is directly related to the number of mismatched epitopes present on that allogenic HLA molecules. Similarly, Peräsaari *et al*. (2008) studied the eplet mismatches defined by HLAMatchmaker program in pediatric heart transplant, and suggested that eplet mismatch number was associate with development of HLA antibody-mediated complications such as coronary artery disease.

Despite the small number of patients studied in our report we could demonstrated PRA Single Antigens results and HLAMachtmaker analysis that choosing the SDS decellularization (PUC solution) process can be an effective alternative for the decrease of the immunogenicity of allograft valve transplant. The clinical significance of these findings requires further investigation.

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4 CONCLUSÃO

A avaliação da resposta imunológica anti-doador após o implante de aloenxertos valvares humanos mostrou que:

- ✓ A resposta imunológica anti-HLA desenvolvida pelos pacientes submetidos ao transplante valvar com aloenxertos criopreservados foi maior que pelos pacientes que receberam implante de aloenxerto descelularizado;
- ✓ Os pacientes submetidos ao transplante com aloenxerto descelularizado pela solução PUC (SDS) apresentaram resposta imunológica reduzida, onde a diferença na formação de anticorpos anti-HLA no pós-tranplante foi significativa ($p=0,015$) para classe II ;
- ✓ Nos pacientes submetidos ao transplante com aloenxertos criopreservados, os possíveis epítopes imunogênicos presentes nas moléculas HLA contra as quais foram formados anticorpos durante o monitoramento pós-transplante são contra as moléculas HLA do doador. Enquanto que nos pacientes submetidos ao transplante com aloenxerto descelularizado os possíveis epítopos imunogênicos encontrados contra as moléculas HLA do doador não foram responsáveis pela reação imunológica desenvolvida;

E ainda,

- ✓ A escolha pelo processo de descelularização a partir da solução PUC (SDS) pode ser uma alternativa efetiva na diminuição da imunogenicidade do aloenxerto valvar.

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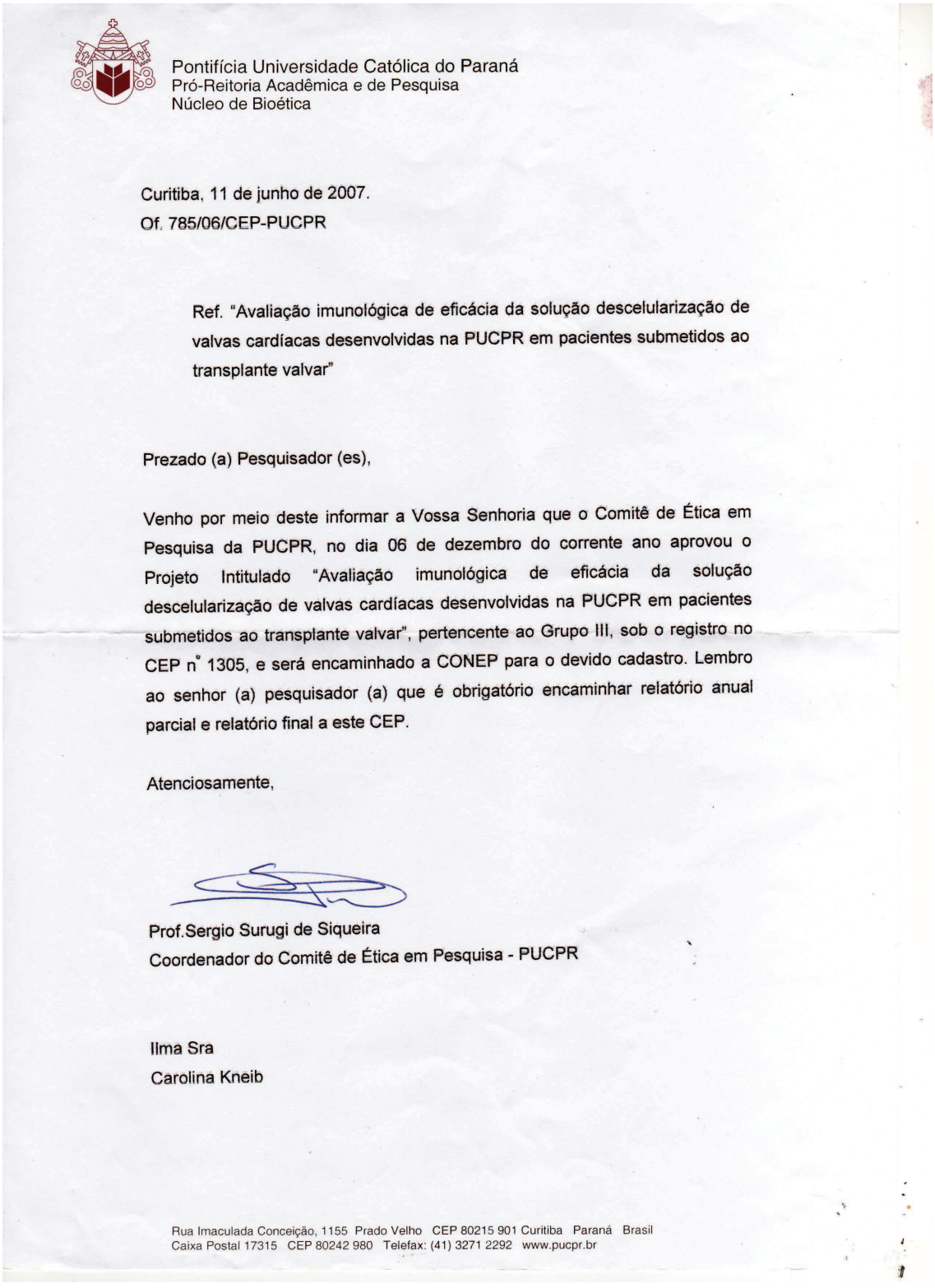
ANEXO

Anexo A - Carta de aprovação do Comitê de Ética



Pontifícia Universidade Católica do Paraná
Pró-Reitoria Acadêmica e de Pesquisa
Núcleo de Bioética

Curitiba, 11 de junho de 2007.
Of. 785/06/CEP-PUCPR

Ref. "Avaliação imunológica de eficácia da solução descelularização de valvas cardíacas desenvolvidas na PUCPR em pacientes submetidos ao transplante valvar"

Prezado (a) Pesquisador (es),

Venho por meio deste informar a Vossa Senhoria que o Comitê de Ética em Pesquisa da PUCPR, no dia 06 de dezembro do corrente ano aprovou o Projeto Intitulado "Avaliação imunológica de eficácia da solução descelularização de valvas cardíacas desenvolvidas na PUCPR em pacientes submetidos ao transplante valvar", pertencente ao Grupo III, sob o registro no CEP nº 1305, e será encaminhado a CONEP para o devido cadastro. Lembro ao senhor (a) pesquisador (a) que é obrigatório encaminhar relatório anual parcial e relatório final a este CEP.

Atenciosamente,

Prof.Sergio Surugi de Siqueira
Coordenador do Comitê de Ética em Pesquisa - PUCPR

Ilma Sra
Carolina Kneib

Rua Imaculada Conceição, 1155 Prado Velho CEP 80215 901 Curitiba Paraná Brasil
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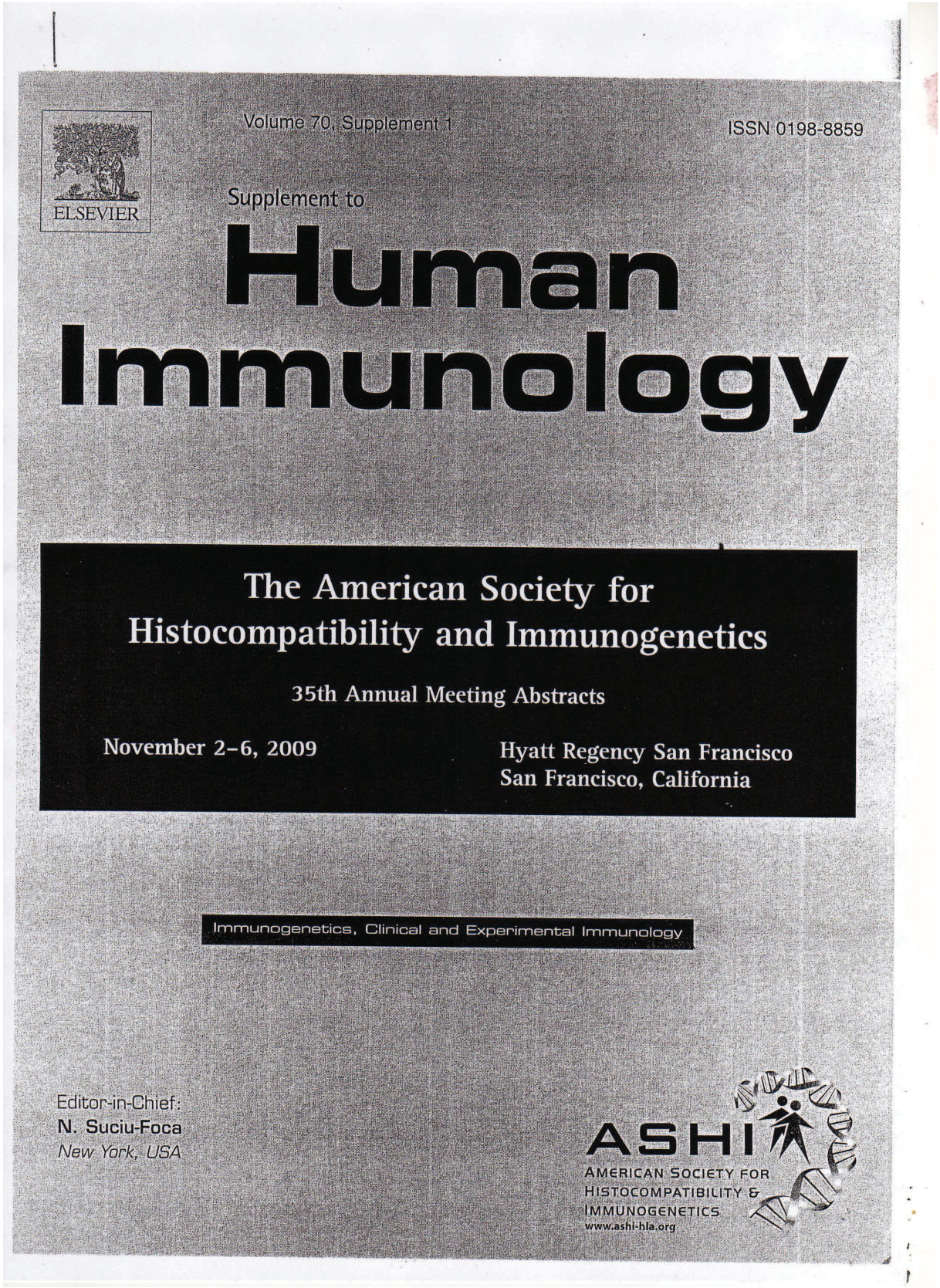
Anexo B – Exposição de Pôster em Congresso – XI Congresso Brasileiro de Transplantes - ABTO (Associação Brasileira de Transplantes de Órgãos) – outubro/2009

Anexo C – Exposição de Pôster em Congresso – ASHI 35th Annual Meetting (The American Society for Histocompatibility and Immunogenetics) – novembro/2009



Anexo D – Exposição de Pôster em Congresso (Abstract) – ASHI 35th Annual Meetting (The American Society for Histocompatibility and Immunogenetics) – novembro/2009

110-P ARE DIFFERENT CYTOKINE GENE POLYMORPHISMS INFLUENCE ON GFR DECLINE AND 5-YEAR KIDNEY GRAFT SURVIVAL. Urszula Siekiera,[3] Jerzy Chudek,[1] Henryk Karkoszka,[1] Leszek Cierpka,[2] Andrzej Wiecek.[1] [1]Department of Nephrology Endocrynology&Metabolic Disease, Medical University of Silesia, Katowice, Poland; [2]Department of General Vascular and Transplantation Surgery, Medical University of Silesia, Katowice, Poland; [3]HLA&Immunogenetics Laboratory, Blood Center, Katowice, Poland.

Aim: The aim of this study was to assess the impact of TNF-a, IL-10, IL-6, and IFN-g TGF-β1,genotypes on GFR and long-term kidney graft outcome.

Methods: Cytokines genotyping was performed in 240 subsequent recipients from January 1998 to December 2002.PCR-SSP method was used for identification: TNF-a at position -308 A/G, IL-10 at positions -1082 A/G, -819 T/C, -592 A/C, IL-6 at position -174 G/C, IFN-g at position +874 T/A and TGFβ1 in codon 10(T/C),25 (G/C). Nineteen patients with primary graft nonfunction were excluded. During 5-year follow up period 17 patients died with functioning kidney graft and 35 patients developed graft failure.Glomerulare filtration rate (eGFR) was estimated based on MDRD equation. The yearly eGFR decline was calculated from 6 months to 5 years period of observation.

Results: IL-6 gene polymorphism had significant impact on kidney graft survival and decline of eGFR. In patients with CC genotype (determining low IL-6 production) 6 out of 68 patients (8.8%) lost kidney graft.In the group with GG and GC genotypes (determining higher IL-6 production) 29 out of 151 (19.2%). The risk of graft loss (hazard ratio) was 2.38 (1.01-4.16); p=0.046 for GG or GC carriers. The frequency of death was similar in both groups (7.3 and 7.9%). eGFR decline tended to be faster in GG or GC [-2.15(-2.83- -1.46) ml/min/year] than CC [-1.26(-2.18- -0.34) ml/min/year]; p=0.16.

Conclusions: IL-6 genotypes of the kidney recipient, determining higher IL-6 constitutional expression, are related to the increased risk of graft lost.

111-P EVALUATION OF HUMORAL IMMUNE RESPONSE TO DONOR HLA AFTER IMPLANTATION OF CRYOPRESERVED VERSUS DECELLULARIZED HUMAN HEART VALVE ALLOGRAFTS. C. Kneib,[1] M. Susin,[1] F. Costa,[2] C. Glehn.[1] [1]Immunogenetics Lab, PUCPR, Curitiba, Parana, Brazil; [2]Santa Casa de Curitiba, PUCPR, Curitiba, Parana, Brazil.

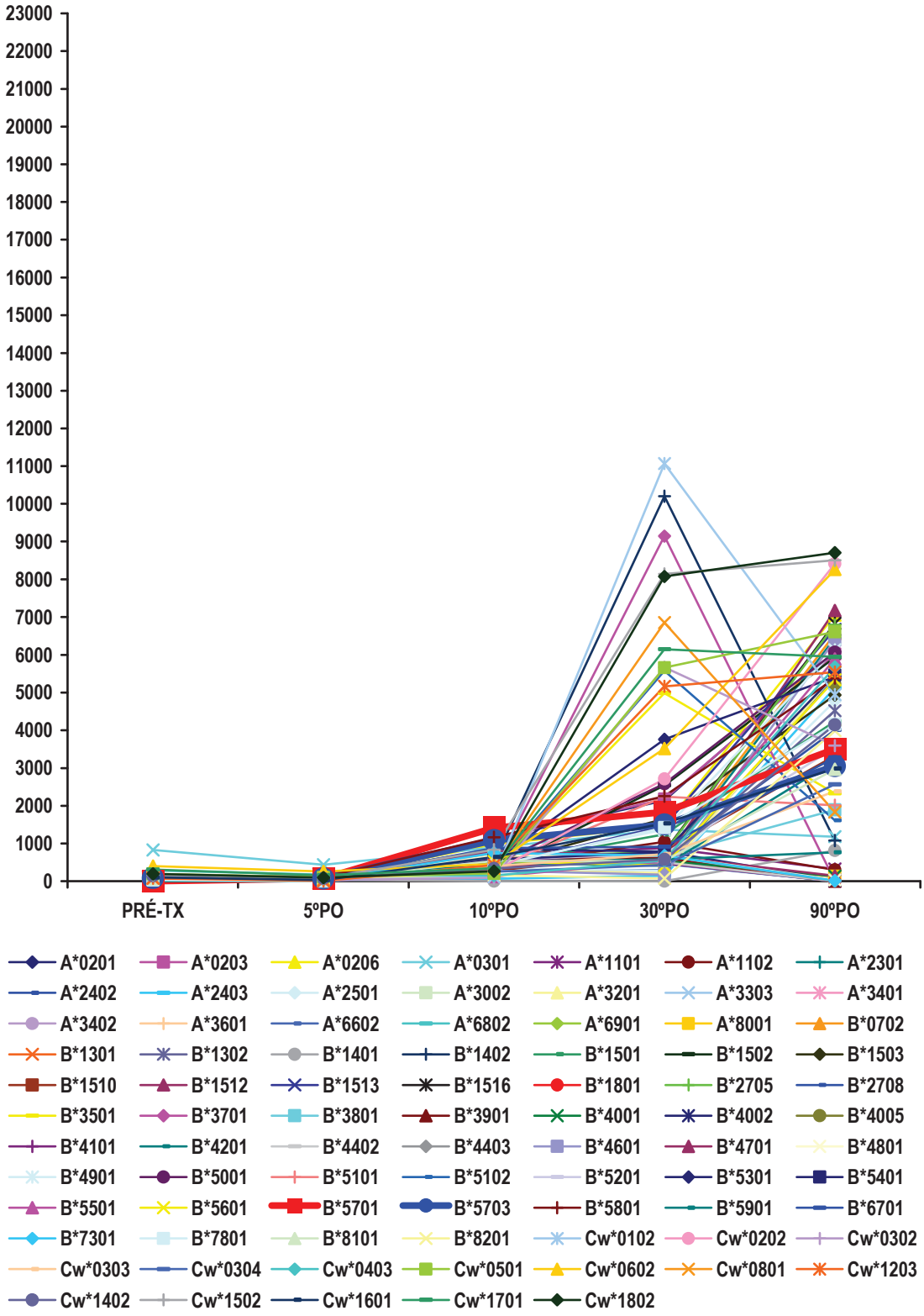
Aim: The aim of this study was to evaluate the efficacy of the SDS (Sodium Dodecyl Sulfate) decellularization process to decrease or prevent the development of humoral antibodies in response to donor allograft valve implant and determine possible immunogenic epitopes considered responsible for antibodies reactivity.

Methods: Sera samples from all recipients were collected before valve replacement and at 5, 10, 30 and 90 days post operatively and stored at -20°C until required. Tests were performed using Luminex LABScreen® Single Antigen Class I and II. Samples that presented donor specific antibodies were analyzed using the software HLAMatchmaker, an algorithm that assesses donor-recipient histocompatibility by it's amino acid configurations, the eplets.

Results: We observed that decellularized grafts elicited significantly lower levels of class I and class II anti-HLA antibody formation after implantation than did cryopreserved allografts. Furthermore, all patients that received cryopreserved allografts presented DSA class I and II within the 3 months of the observation period. On the other hand, in the decellularized allografts group, two patients of six produced anti-HLA antibodies, including class I DSA. Differently of the cryopreserved graft group, the anti-HLA antibodies detected in post-transplant serum of decellularized patients didn't share eplets with donor-specific antibodies, leading us to believe in different sources of sensitization, e.g. transfusion.

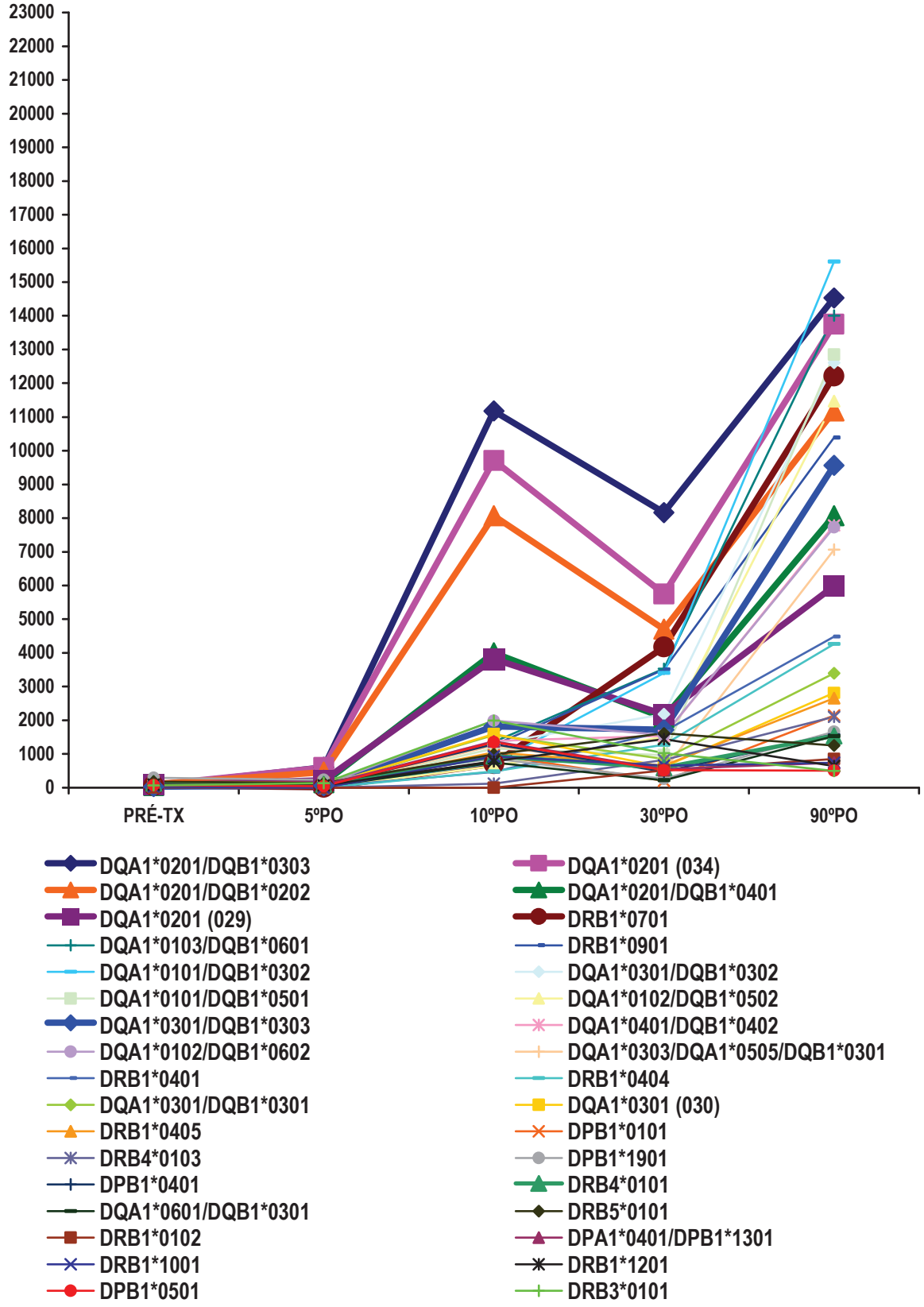
Conclusions: Our findings can demonstrate that choosing decellularized homografts in valve transplant is an effective alternative for the decrease of valve transplant immunological response.

Anexo I – Comportamento dos anticorpos anti-HLA classe I - PAC1C grupo CRIO



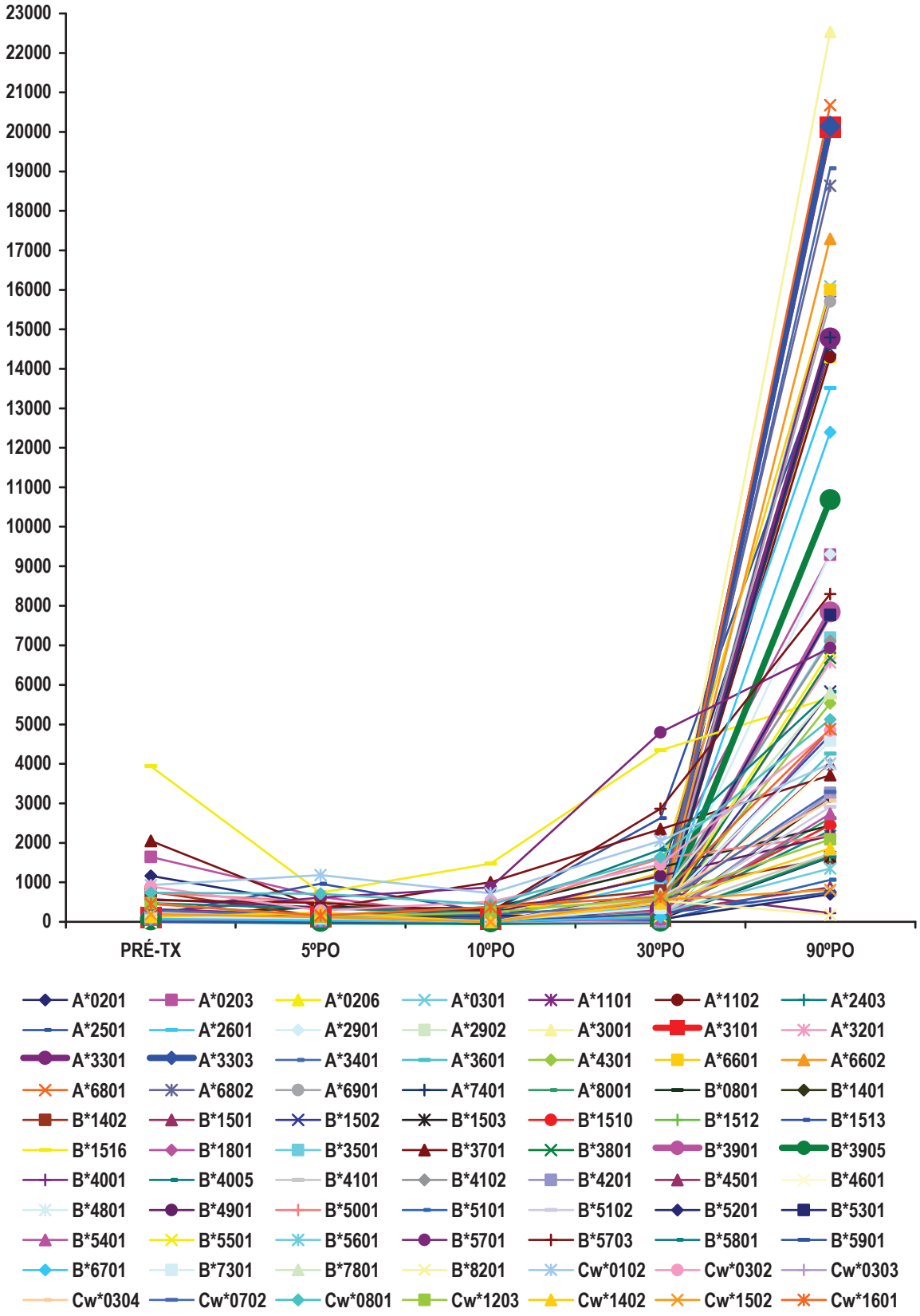
**Anticorpos anti-doador de classe I: B*5701, B*5703 (LINHAS EM NEGRITO)

Anexo J - Comportamento dos anticorpos anti-HLA classe II - PAC1C grupo CRIO



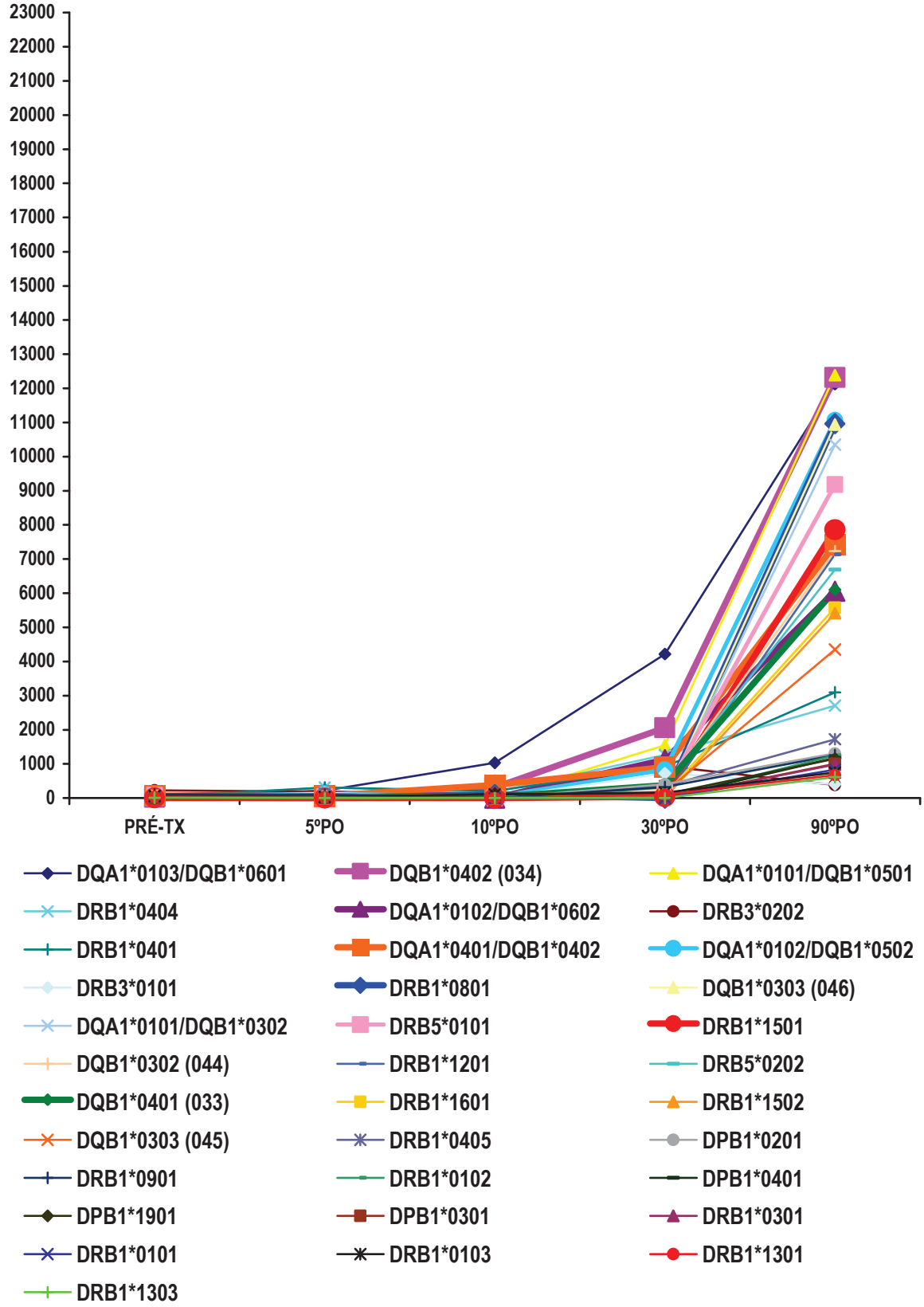
**Anticorpos anti-doador classe II: DRB1*0701, DRB4*0101, DQB1*0202, DQB1*0303, DQA1*0201 (LINHAS EM NEGRITO)

Anexo K - Comportamento dos anticorpos anti-HLA classe I - PAC2C grupo CRIO



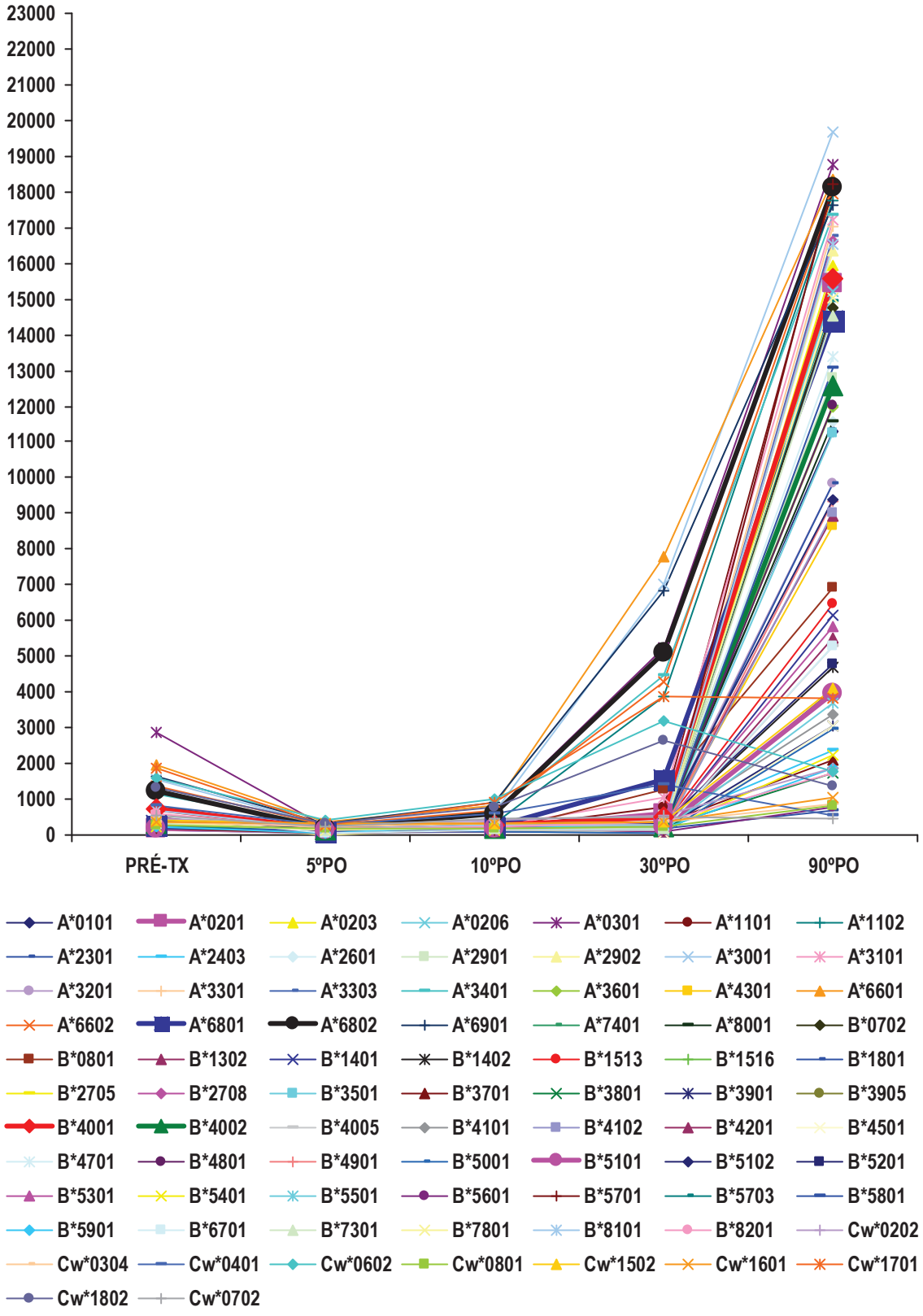
**Anticorpos anti-doador classe I: A*3101, A*3301/03, B*3901/05 (LINHAS EM NEGRITO)

Anexo L - Comportamento dos anticorpos anti-HLA classe II - PAC2C grupo CRIO



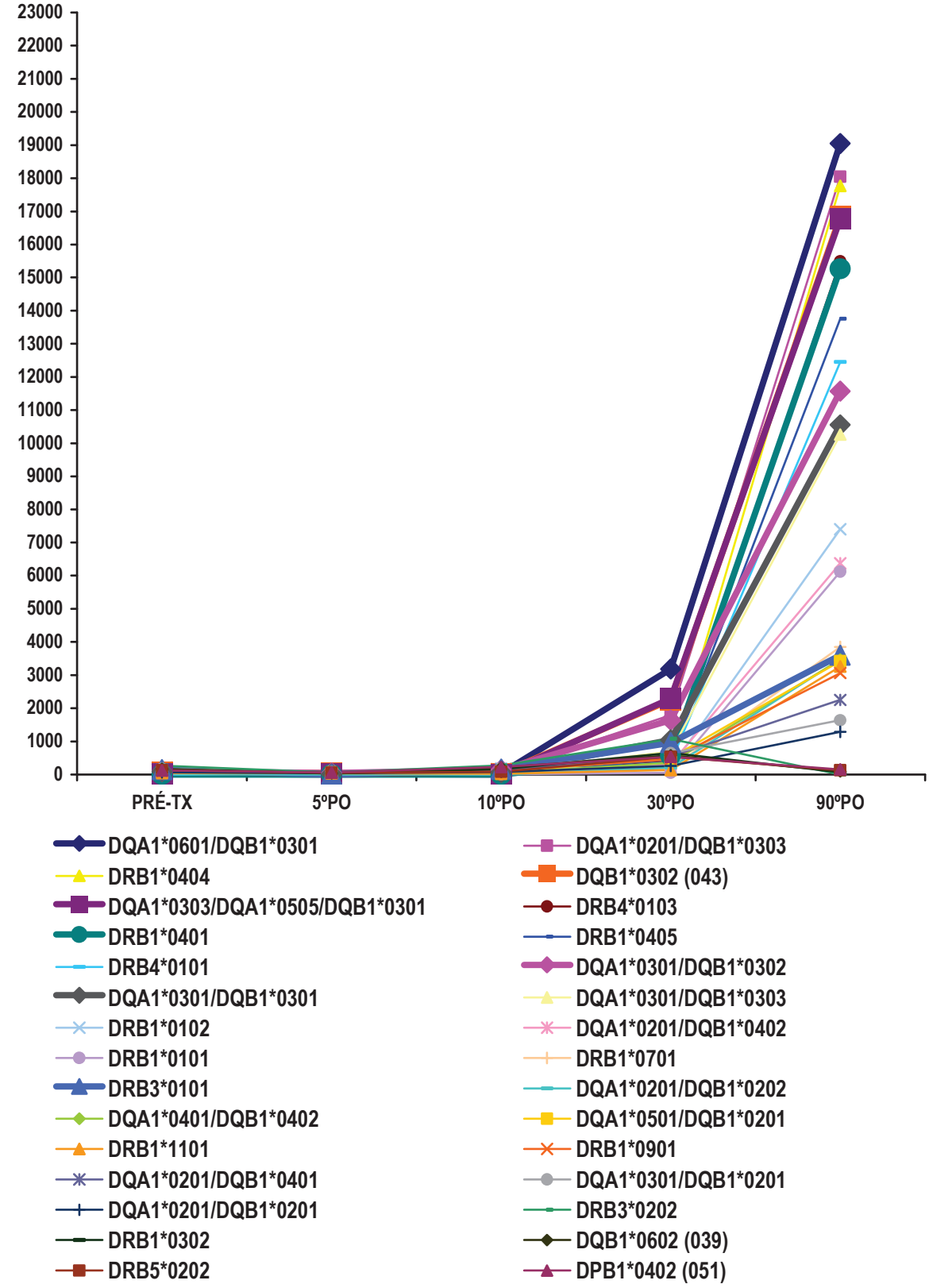
**Anticorpos anti-doador classe II: DRB1*0801, DRB1*1501, DRB5*0101, DQB1*0402, DQA1*0102, DQA1*0401 (LINHAS EM NEGRITO)

Anexo M - Comportamento dos anticorpos anti-HLA classe I - PAC3C grupo CRIO



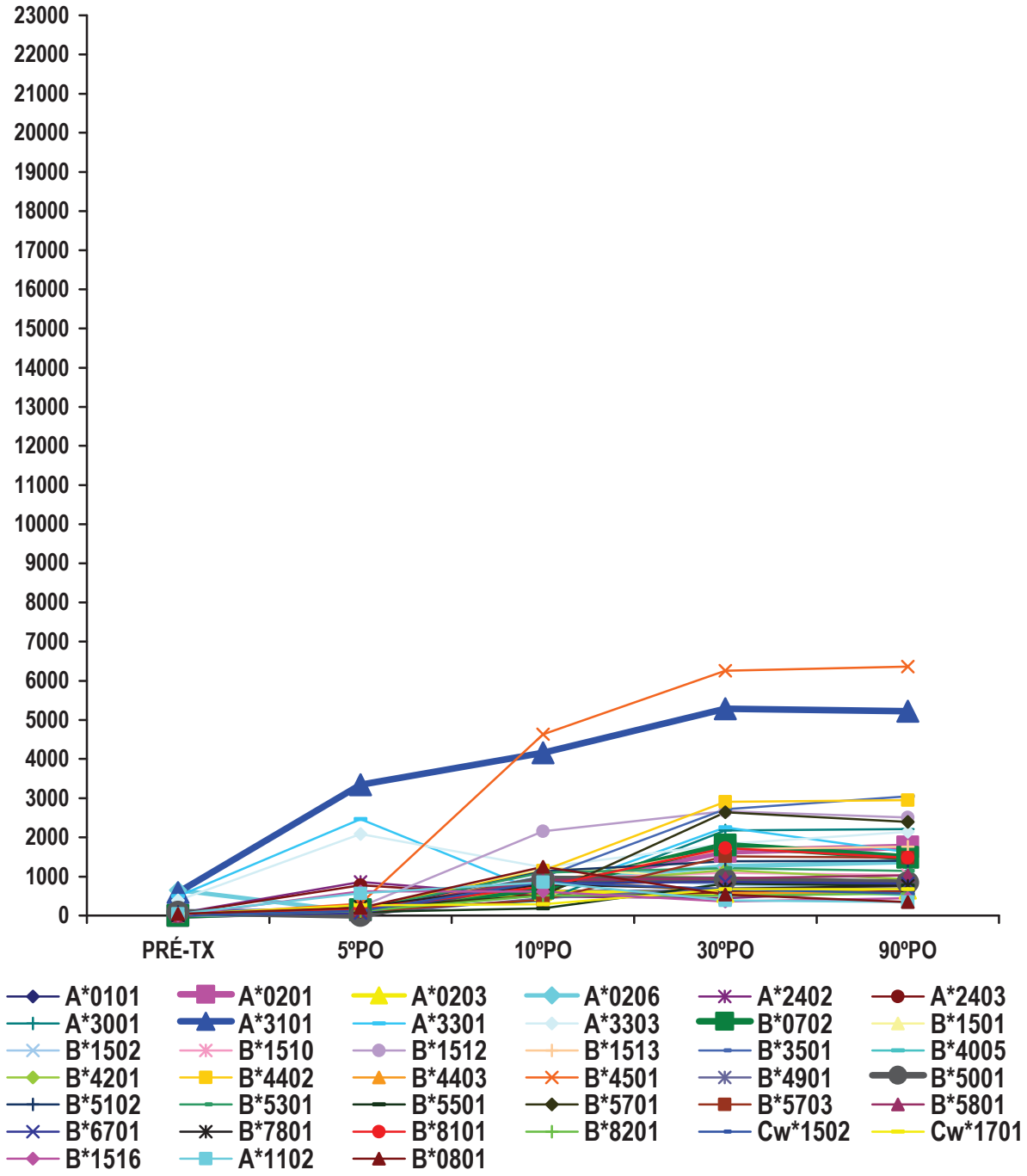
**Anticorpo anti-HLA classe I: A*0201/03/06, A*6801/02, B*4001/02, B*5101/02 (LINHAS EM NEGRITO)

Anexo N - Comportamento dos anticorpos anti-HLA classe II - PAC3C grupo CRIO



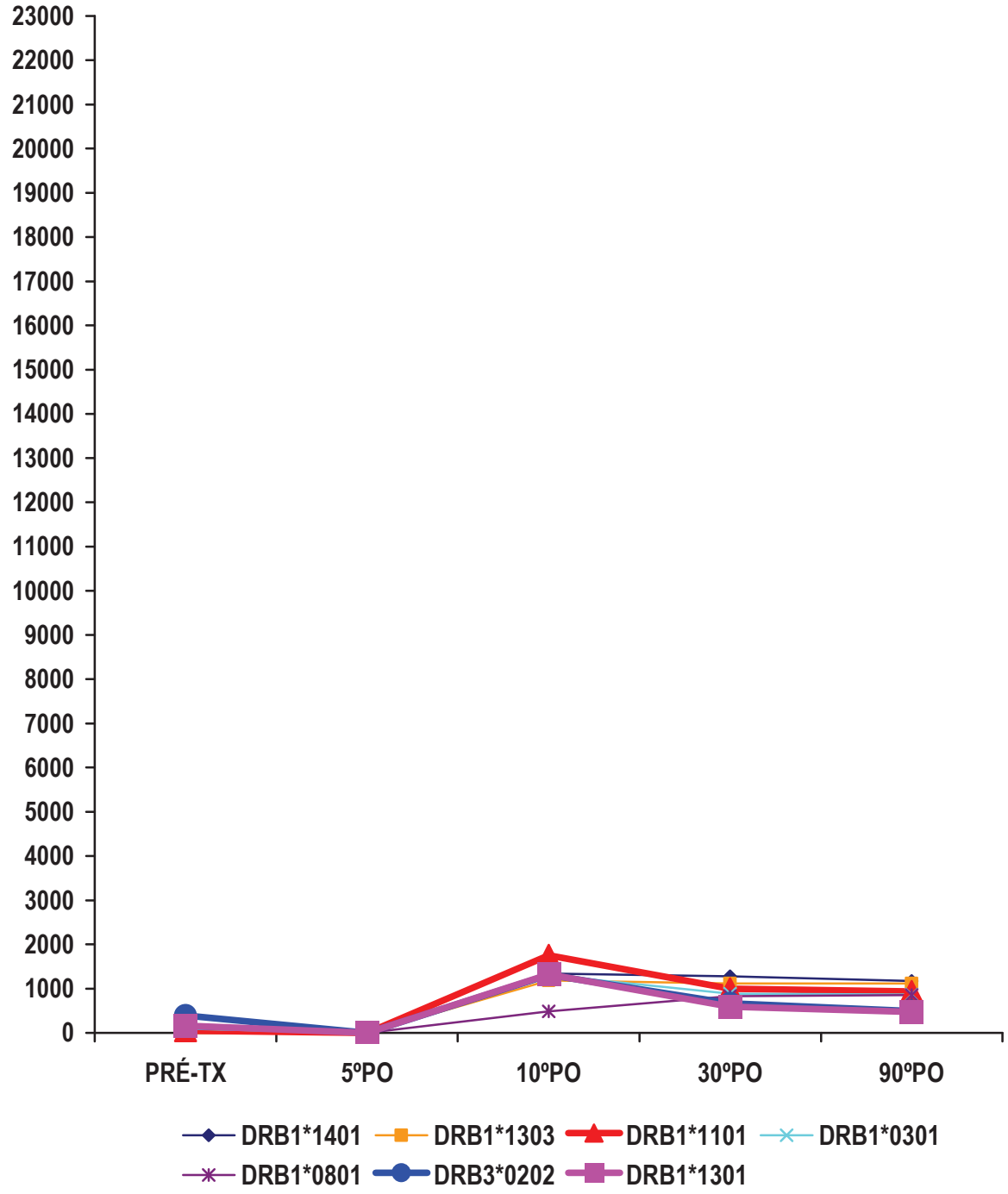
**Anticorpos anti-doador classe II: DRB3*0101, DRB4*0101, DQB1*0301 (LINHAS EM NEGRITO)

Anexo O - Comportamento dos anticorpos anti-HLA classe I - PAC4C grupo CRIO



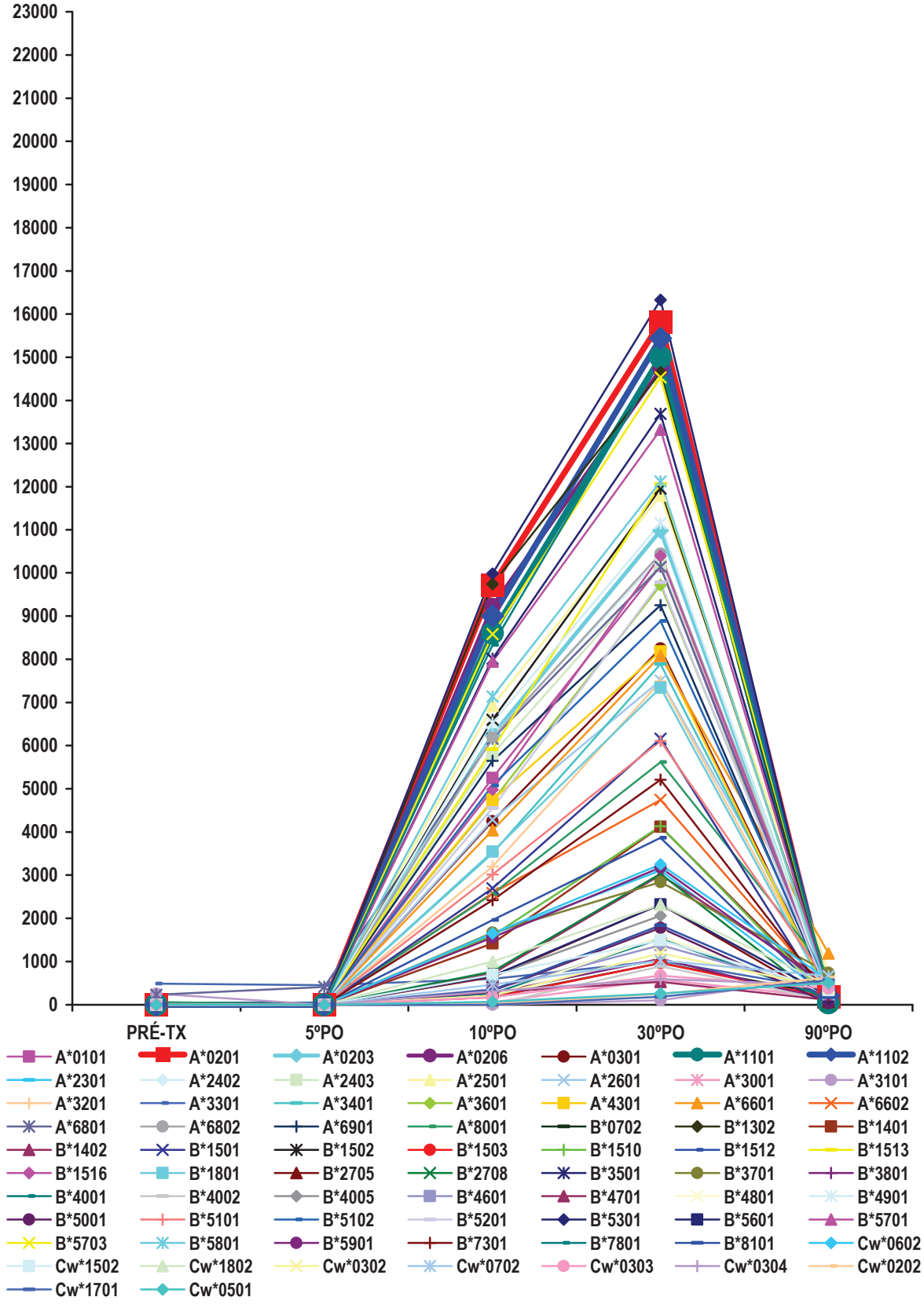
**Anticorpos anti-doador classe I: A*0201/03/06, A*3101, B*0702, B*5001 (LINHAS EM NEGRITO)

Anexo P - Comportamento dos anticorpos anti-HLA classe II - PAC4C grupo CRIO



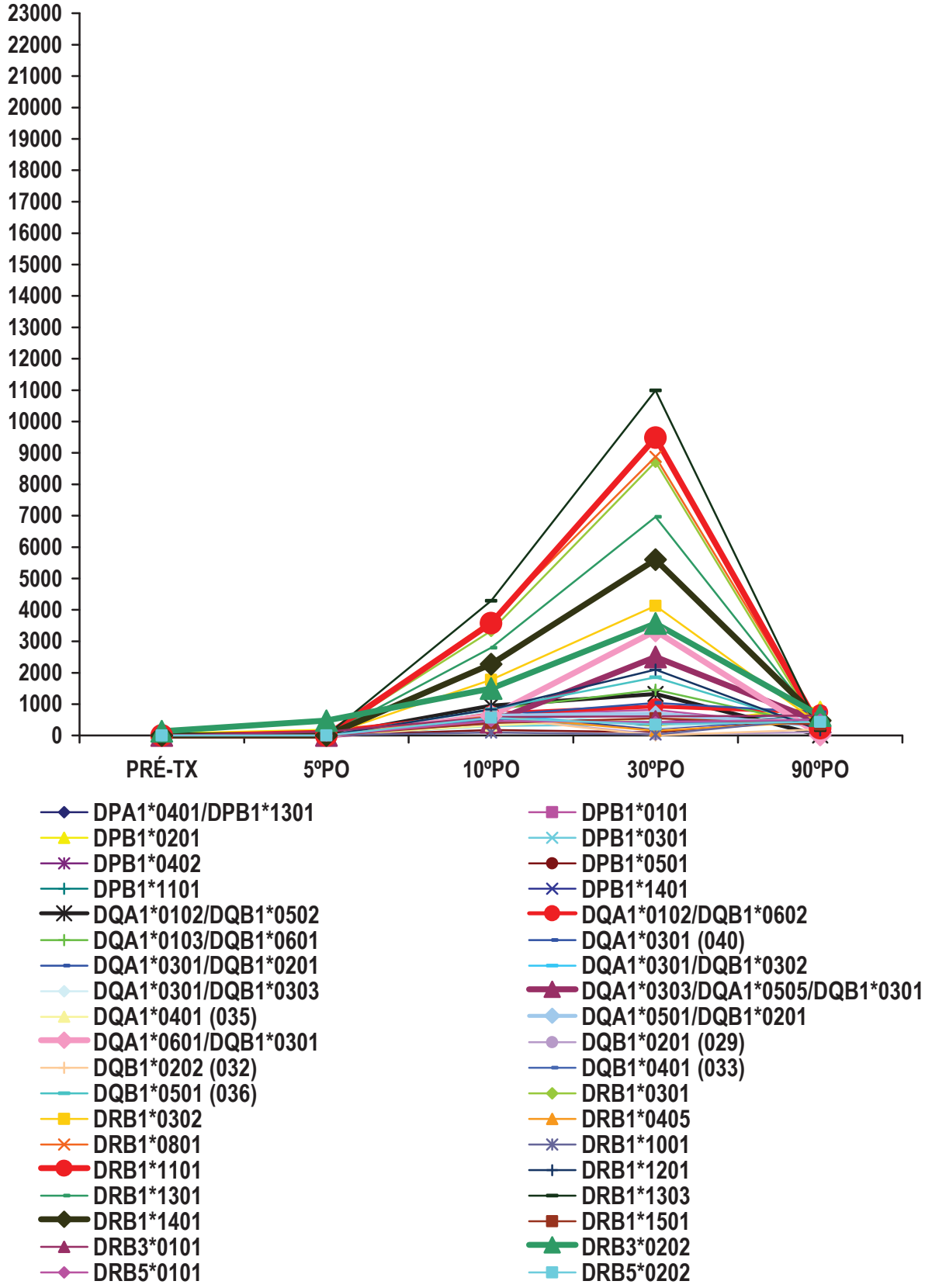
**Anticorpos anti-doador classe II: DRB1*1101, DRB1*1301, DRB3*0202.

Anexo Q - Comportamento dos anticorpos anti-HLA classe I - PAC5C grupo CRIO



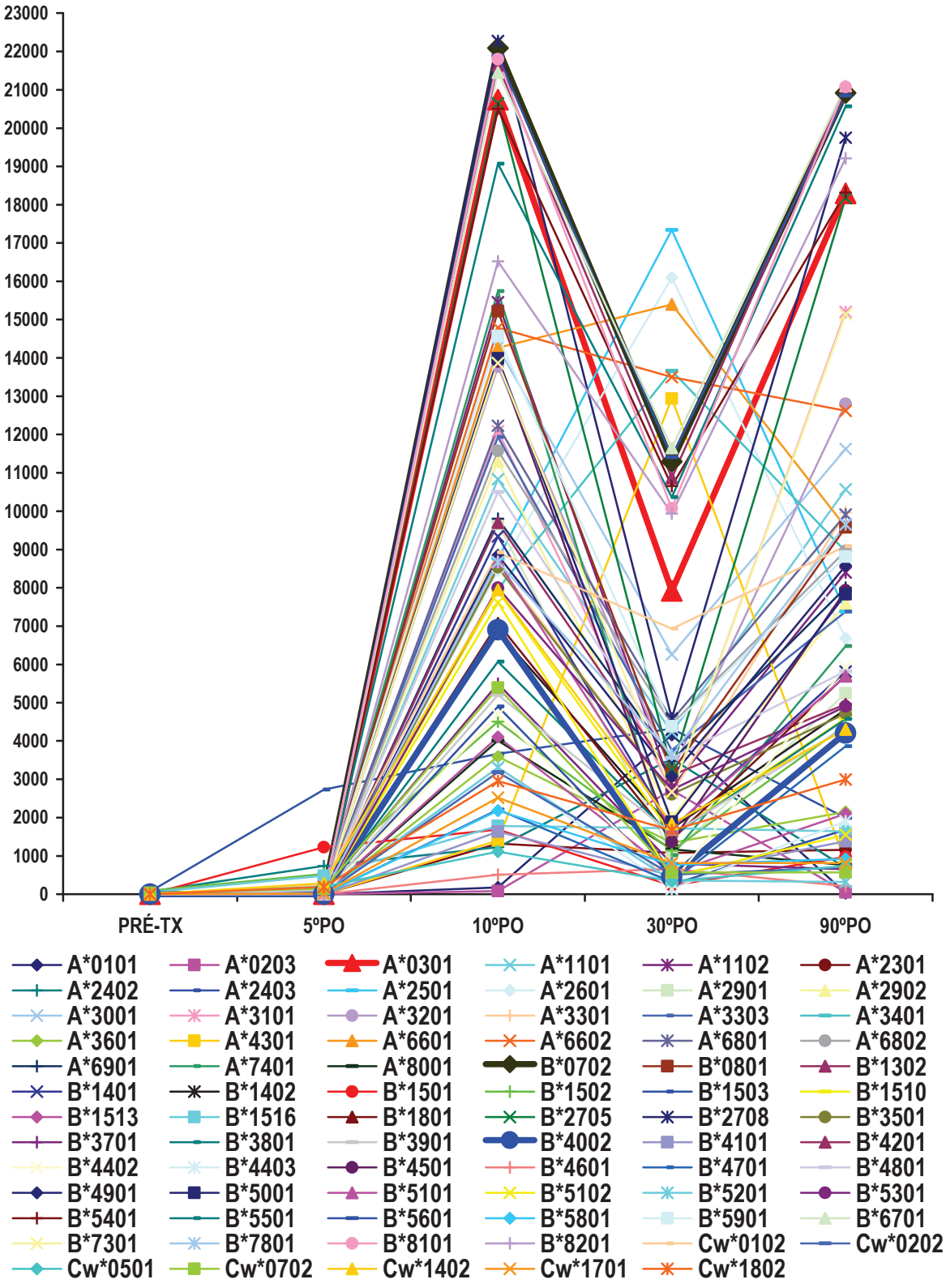
**Anticorpos anti-doador classe I: A*0201/03/06, A*1101/02 (LINHAS EM NEGRITO)

Anexo R - Comportamento dos anticorpos anti-HLA classe I - PAC5C grupo CRIO



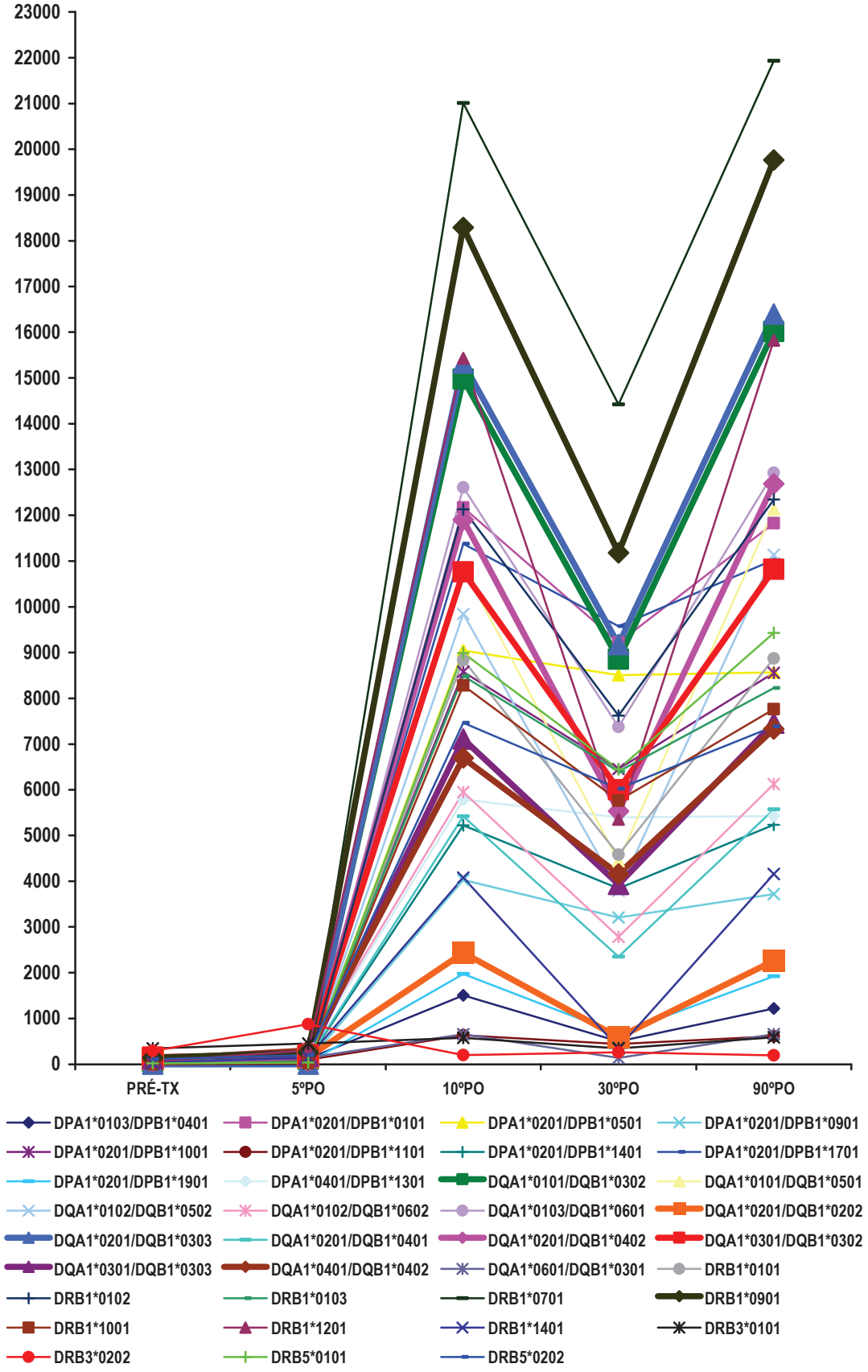
**Anticorpos anti-doador classe II: DRB1*1101, DRB1*1401, DRB3*0202, DQA1*0102, DQA1*0501, DQB1*0301, DQB1*0502 (LINHAS EM NEGRITO)

Anexo S - Comportamento dos anticorpos anti-HLA classe I - PAC6C grupo CRIO.



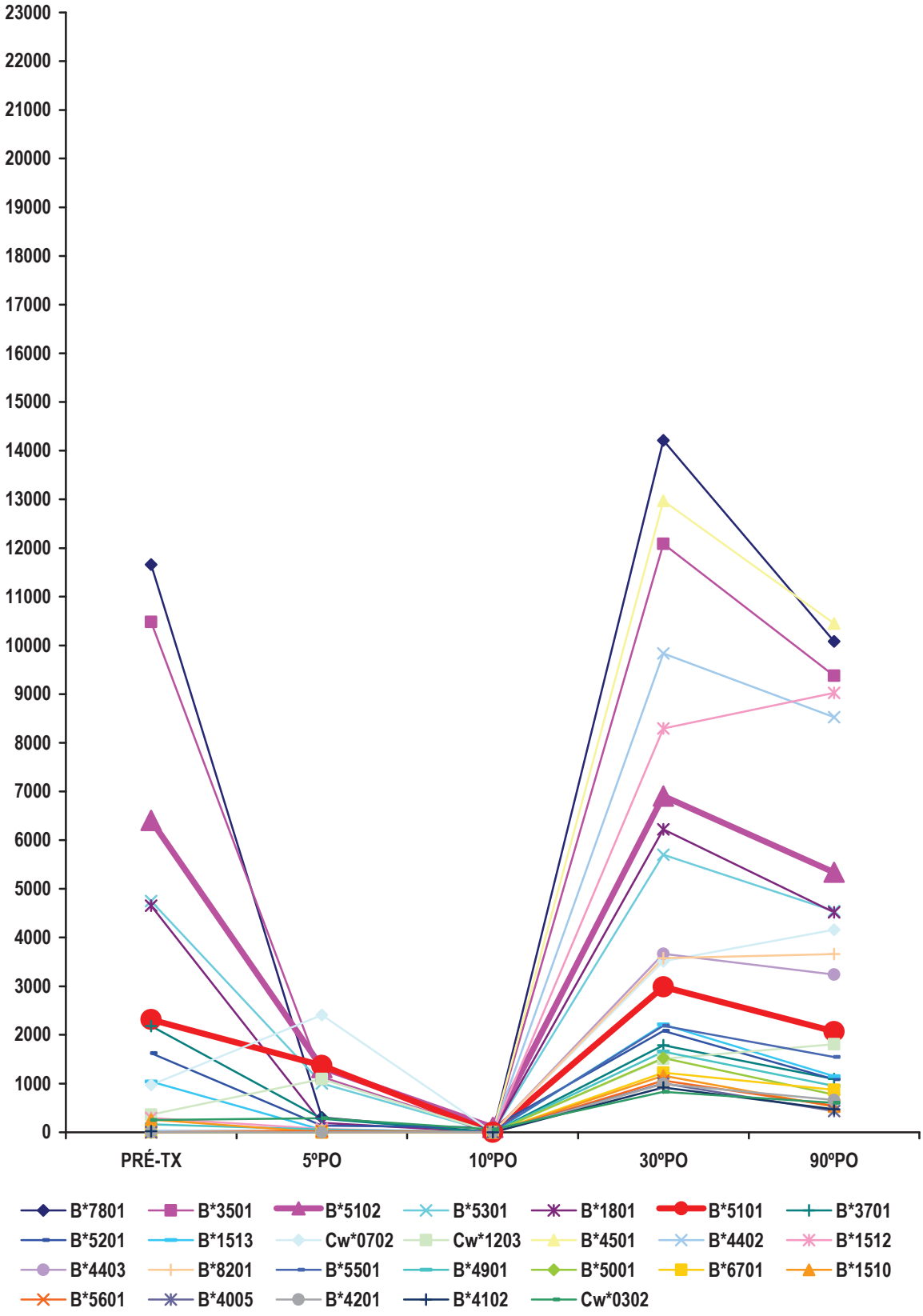
**Anticorpos anti-doador classe I: A*0301, B*0702 (LINHA EM NEGRITO)

Anexo T - Comportamento dos anticorpos anti-HLA classe II - PAC6C grupo CRIO



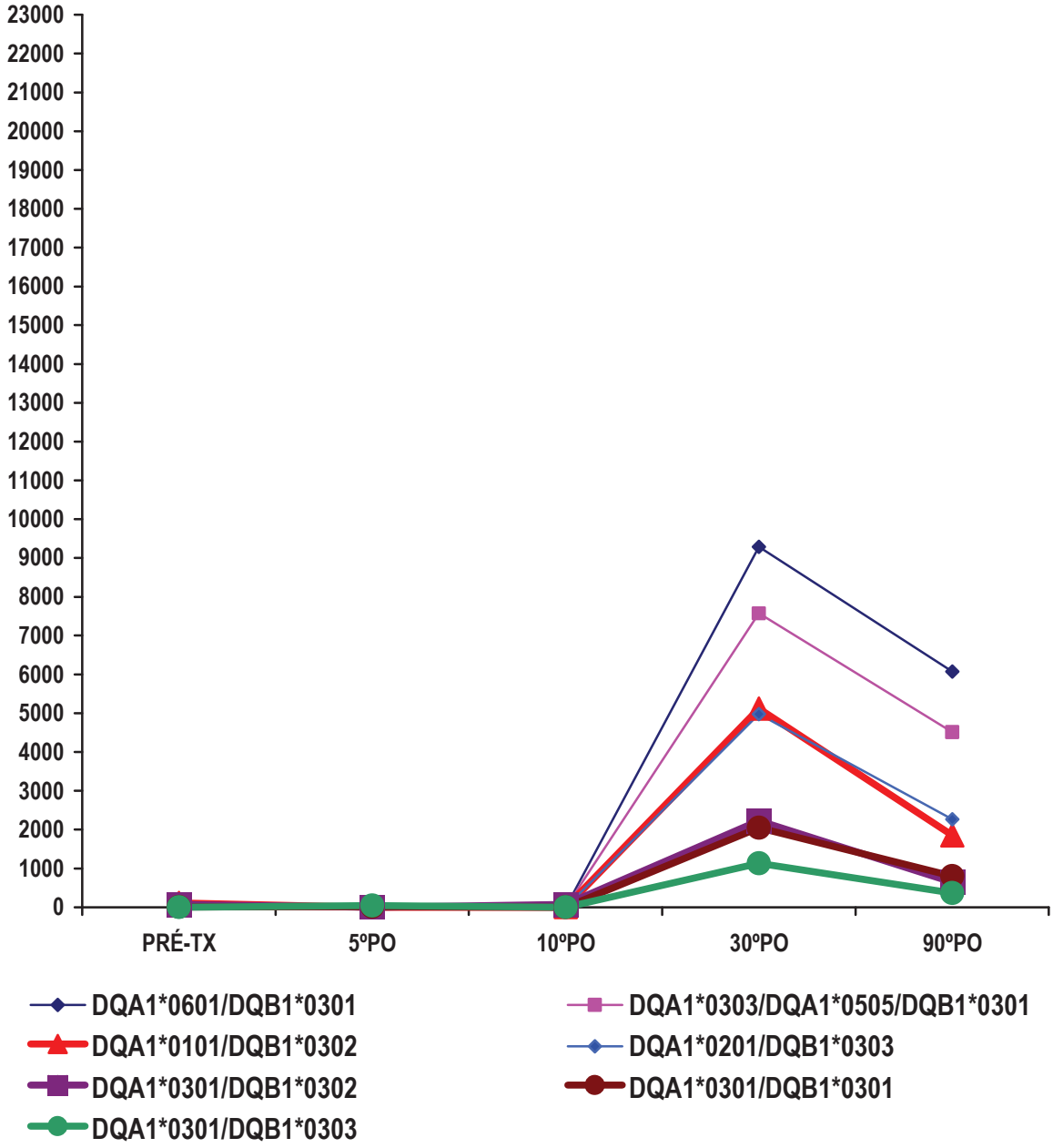
**Anticorpos anti-doador classe II: DRB1*0901, DQB1*0302, DQB1*0402 (LINHAS EM NEGRITO)

Anexo U - Comportamento dos anticorpos anti-HLA classe I - PAC1D grupo DECEL



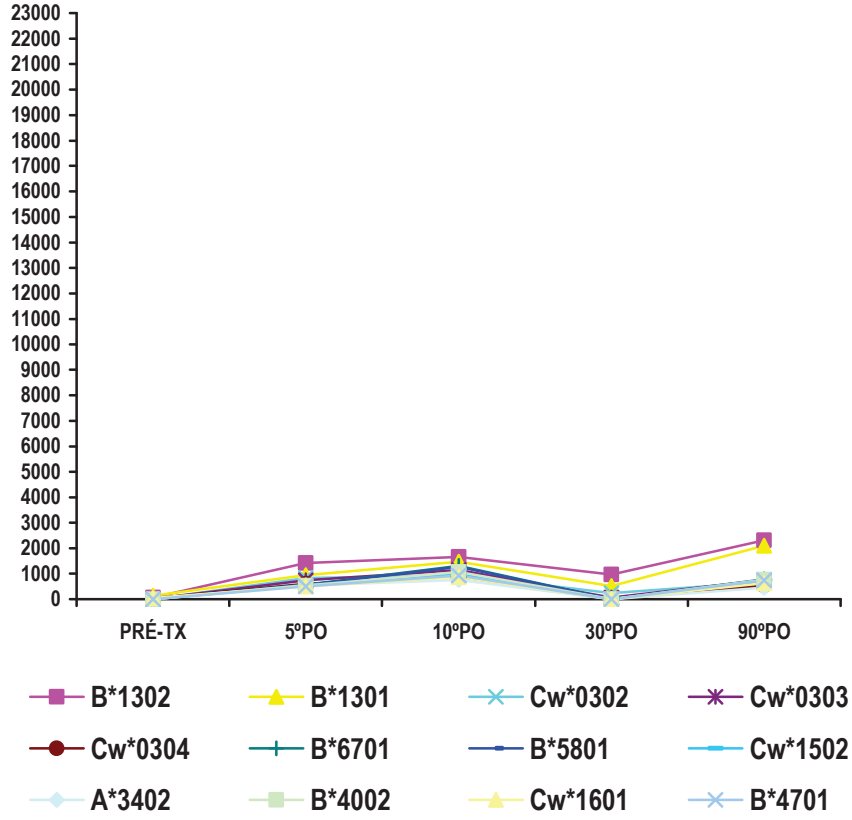
**Anticorpos anti-doador classe I: B*5101/02 (LINHAS EM NEGRITO)

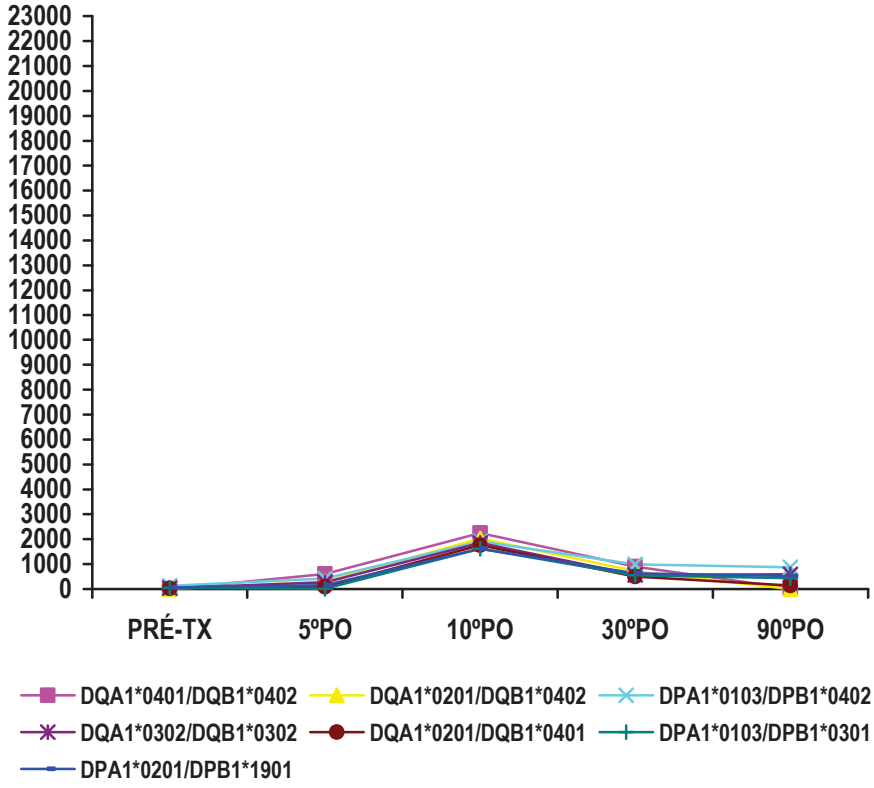
Anexo V - Comportamento dos anticorpos anti-HLA classe II - PAC1D grupo DECEL



**Anticorpos anti-doador classe II: DQA1*0301, DQA1*0302, DQB1*0302 (LINHAS EM NEGRITO)

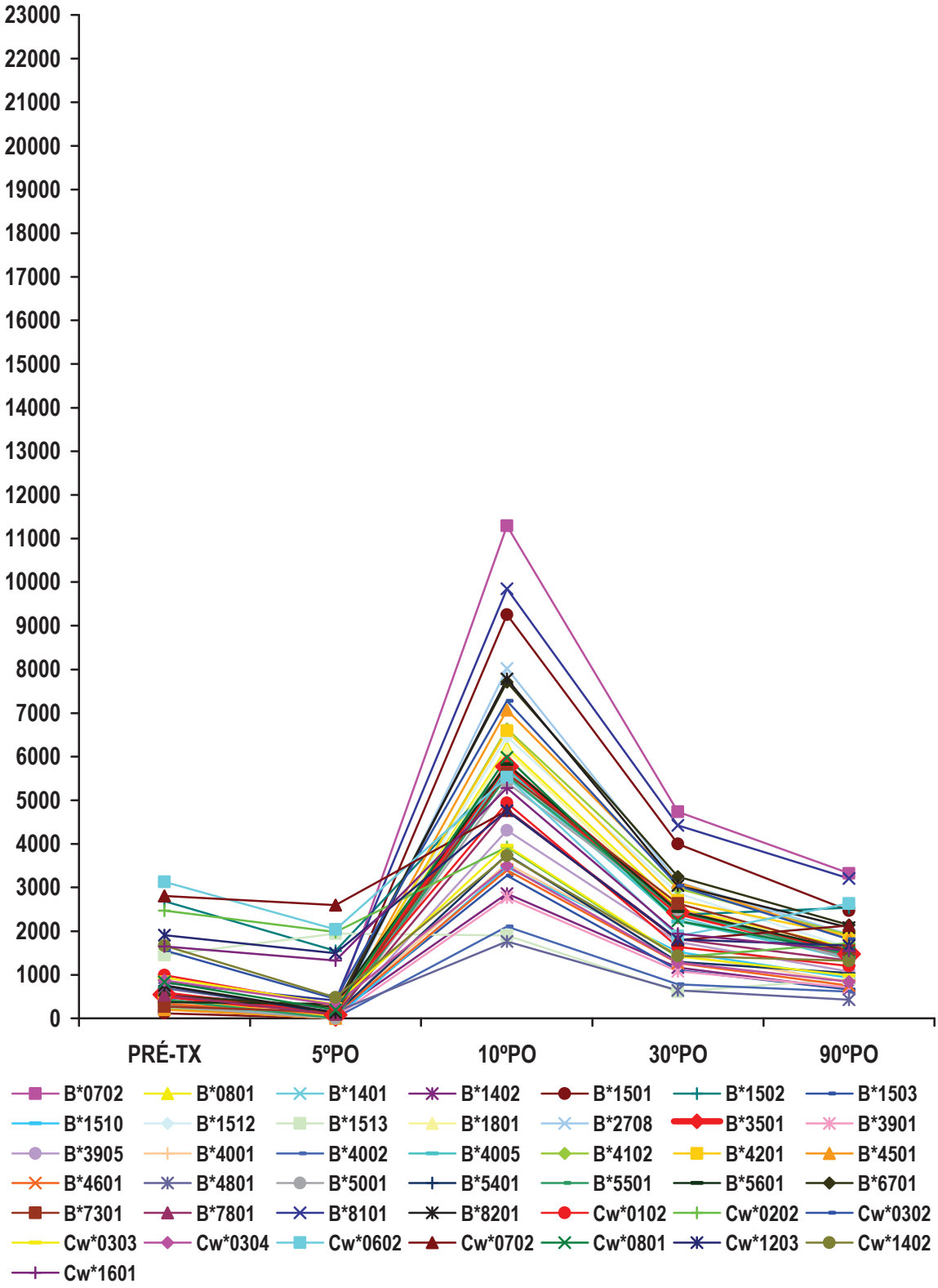
Anexo X - Comportamento dos anticorpos anti-HLA classe I e II - PAC2D grupo DECEL





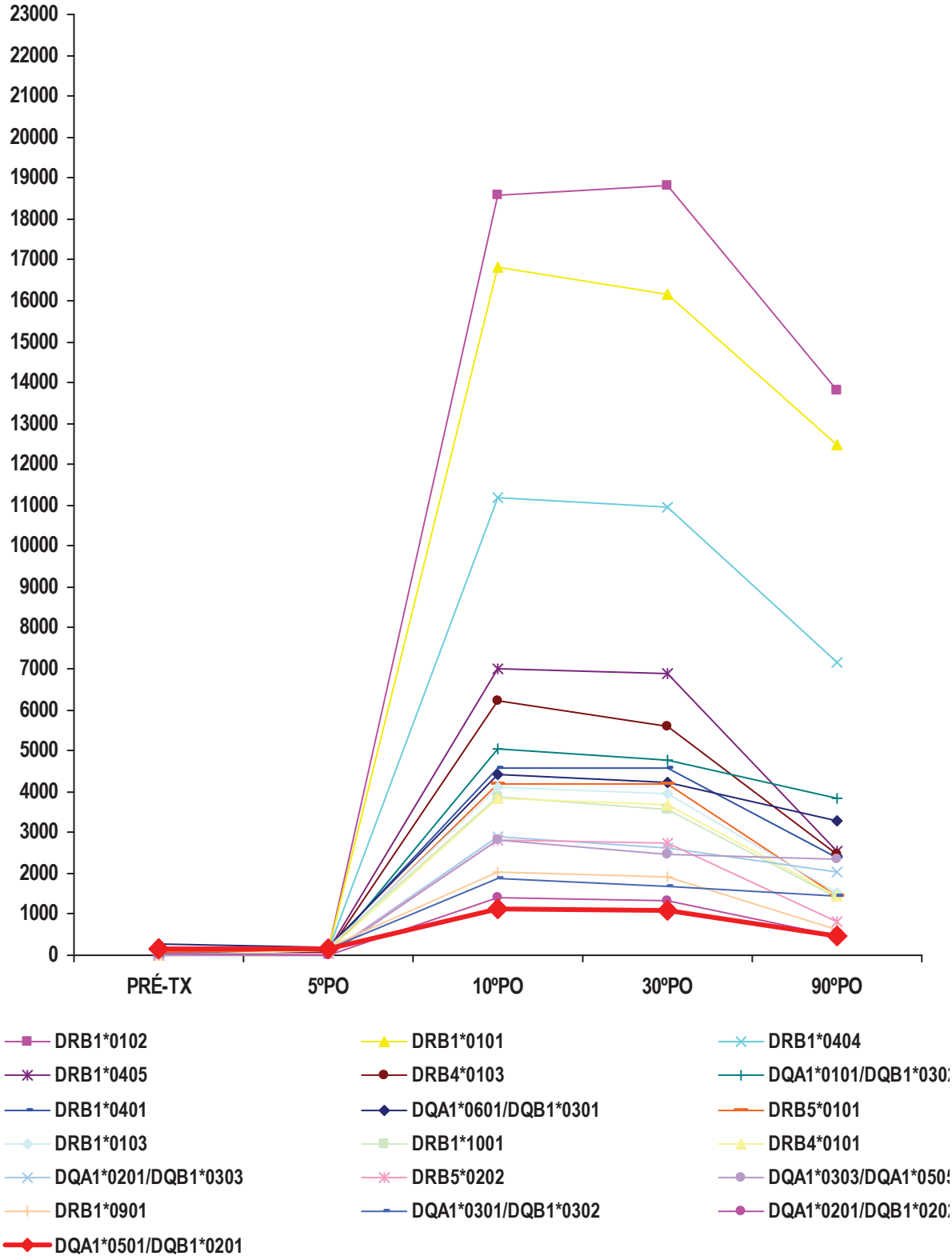
**SEM ANTICORPOS ANTI-DOADOR.

Anexo W - Comportamento dos anticorpos anti-HLA classe I - PAC3D grupo DECEL



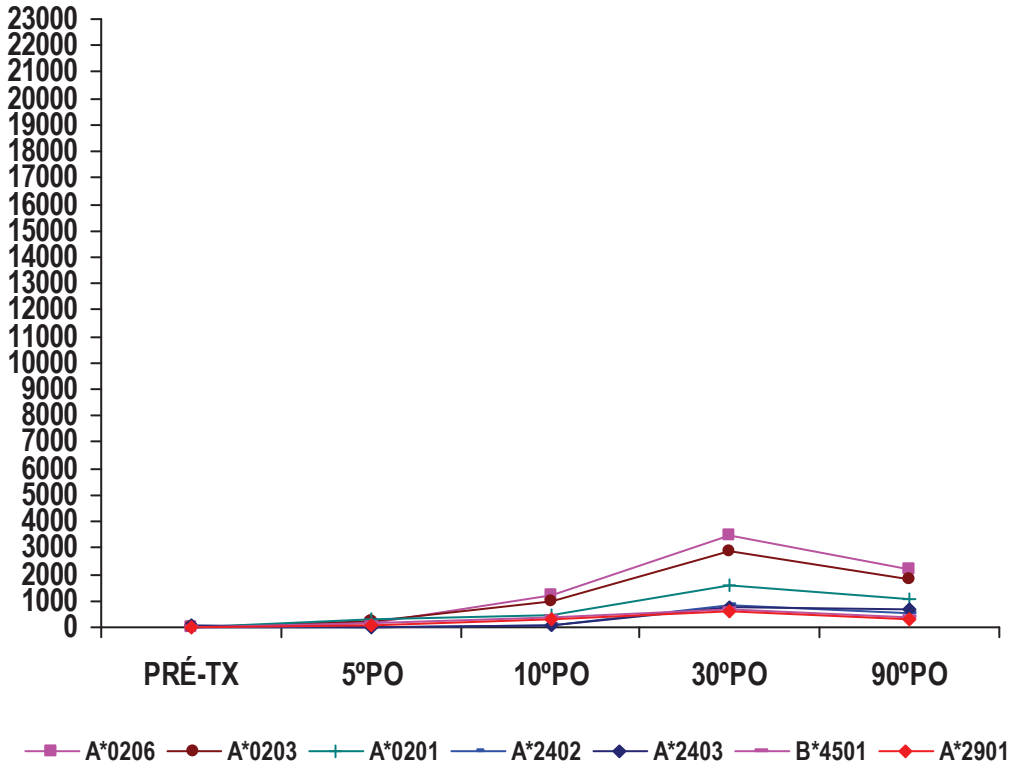
**Anticorpos anti-doador classe I: B*3501 (LINHAS EM NEGRITO)

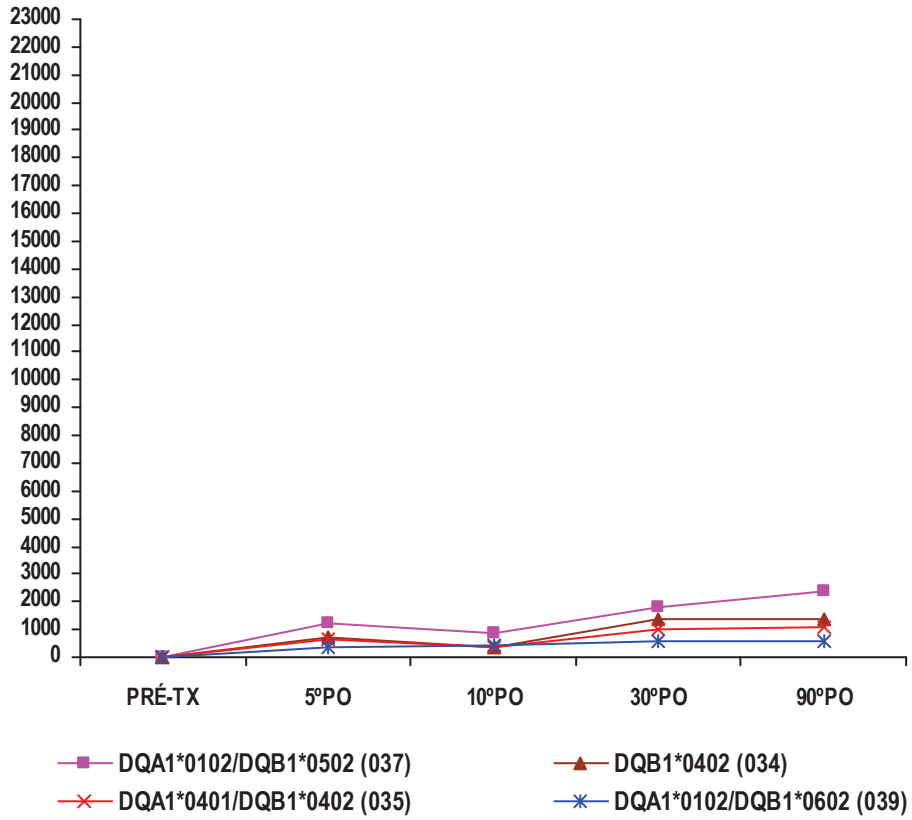
Anexo Y - Comportamento dos anticorpos anti-HLA classe II - PAC3D grupo DECEL



**SEM ANTICORPOS ANTI-DOADOR.

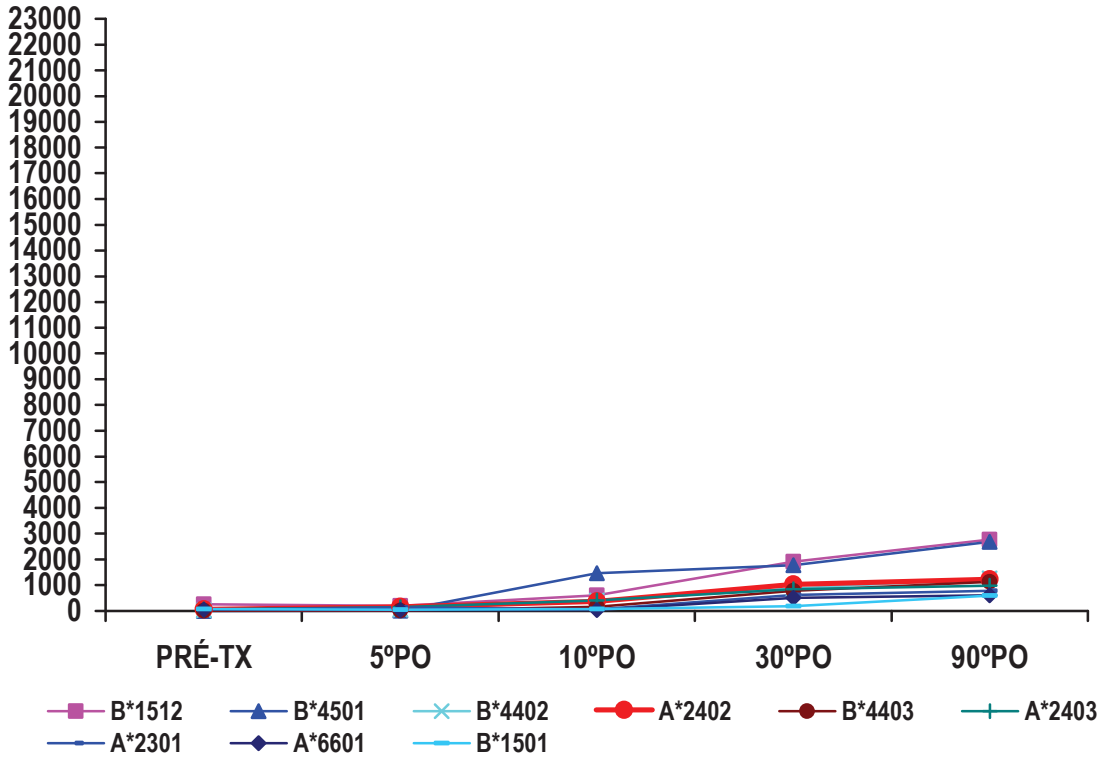
Anexo Z - Comportamento dos anticorpos anti-HLA classe I e II - PAC4D grupo DECEL

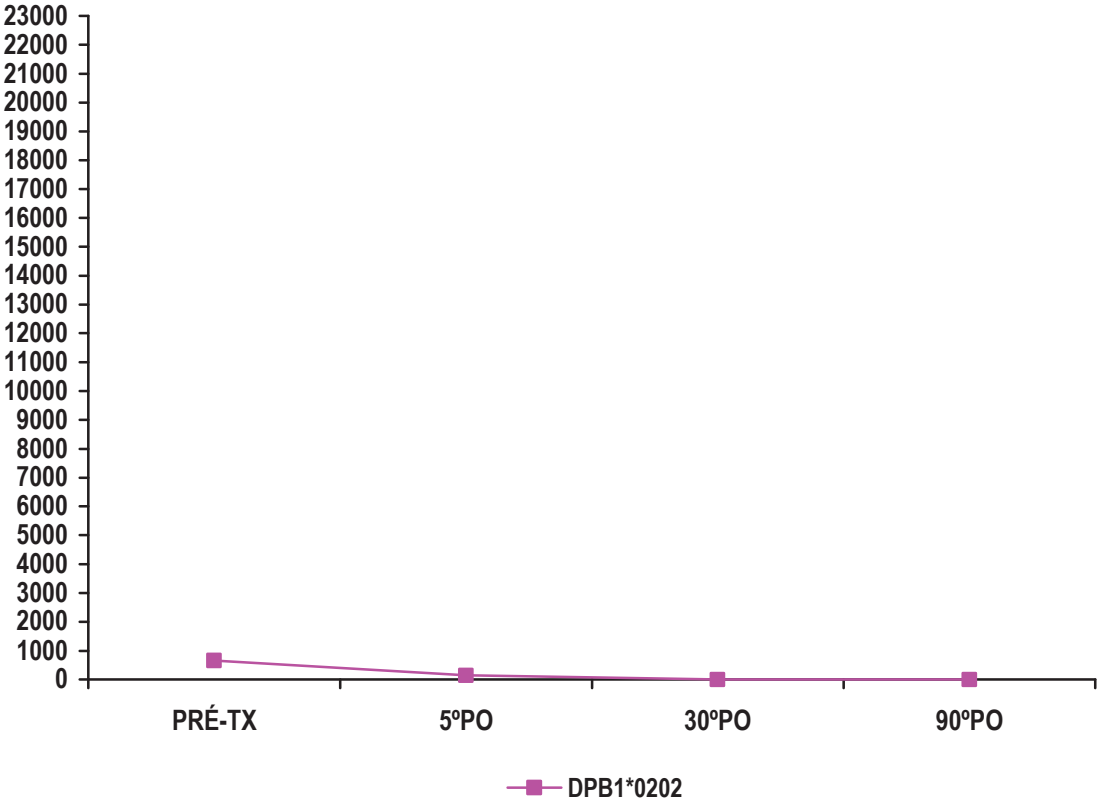




**Anticorpos anti-doador classe I e II: A*0201/03/06, DQA1*0102, DQB1*0502 (LINHAS EM NEGRITO)

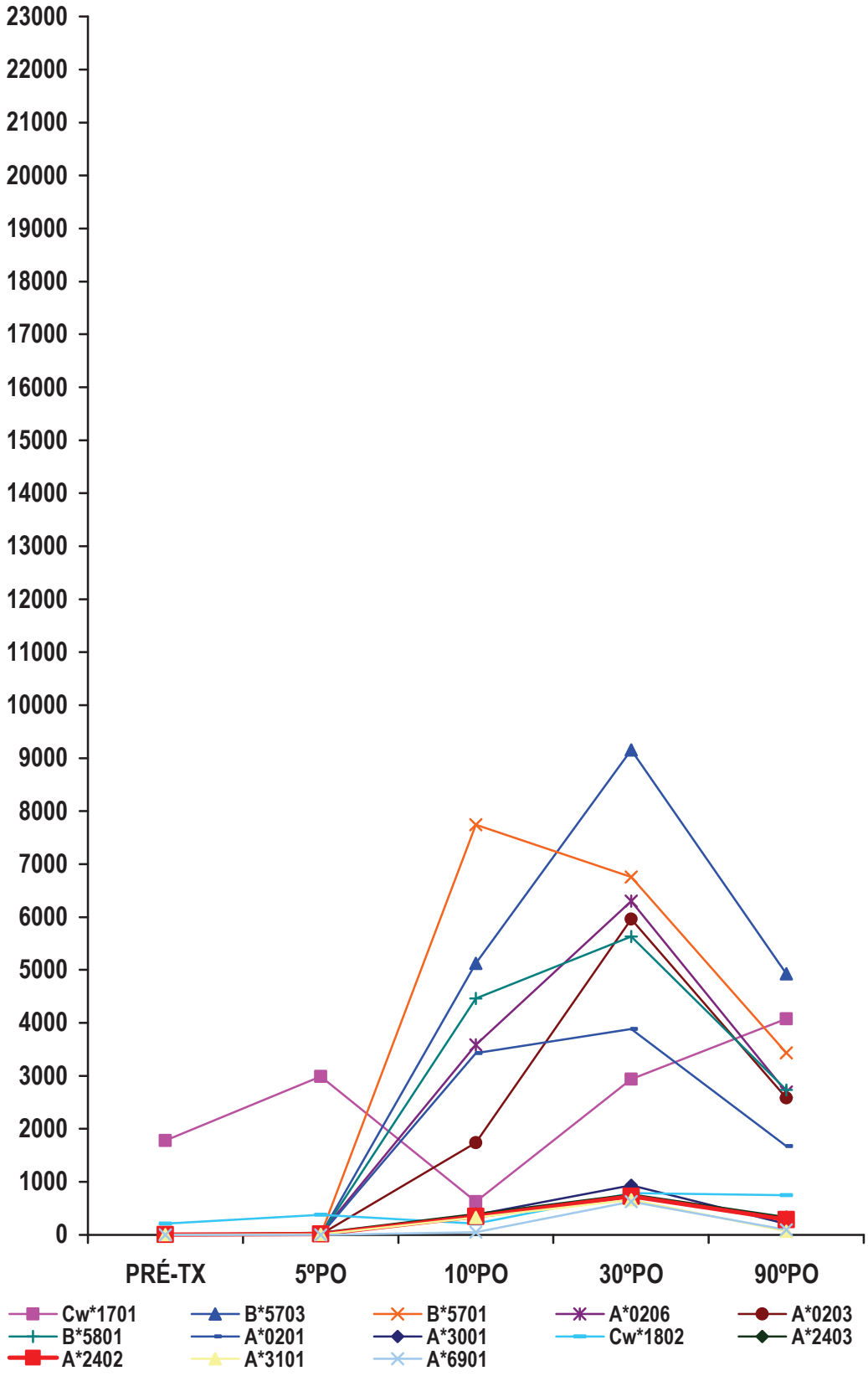
Anexo AA - Comportamento dos anticorpos anti-HLA classe I e II - PAC5D grupo DECEL





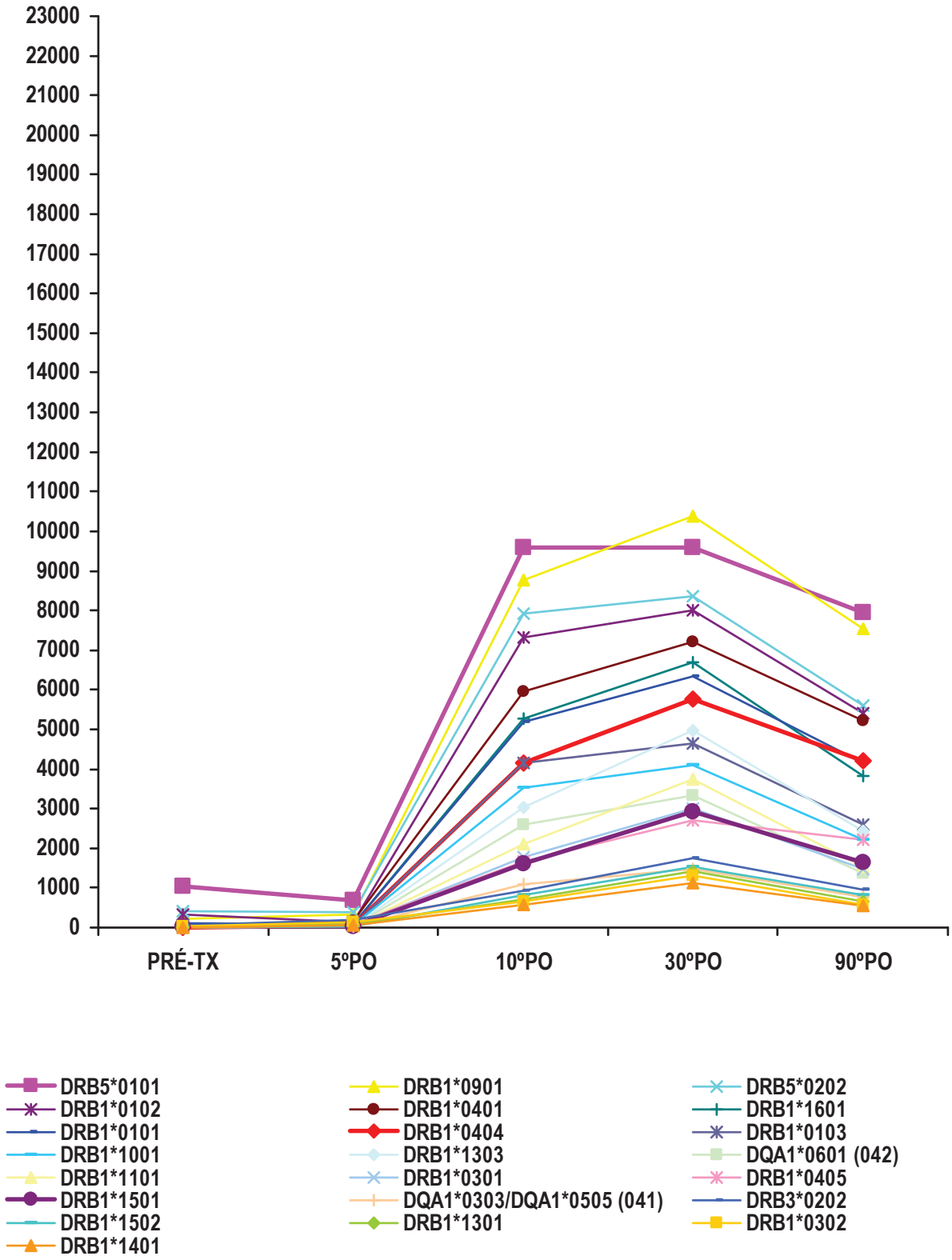
**Anticorpos anti-doador classe I: A*2402/03 (LINHAS EM NEGRITO) – SEM ANTICORPOS ANTI-DOADOR CLASSE II

Anexo AB - Comportamento dos anticorpos anti-HLA classe I - PAC6D grupo DECEL



**Anticorpos anti-doador classe I: A*2402/03 (LINHAS EM NEGRITO)

Anexo AC - Comportamento dos anticorpos anti-HLA classe II - PAC6D grupo DECEL



**Anticorpos anti-doador classe II: DRB1*1501, DRB5*0101 (LINHAS EM NEGRITO)