Appendix A

FDA Systematic Literature Review: HLA Devices

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1. Methods

FDA conducted a systematic literature review to assess the safety and effectiveness of FDA cleared HLA devices. The safety and effectiveness of HLA devices relates to the accuracy of HLA test results used to aid donor and recipient matching and disease diagnosis. Articles examining HLA device analytical performance are included in this review, as analytical performance directly correlates to the safe and effective use of the HLA device.

We recognize that in addition to HLA, other factors could impact on clinical outcome in the transfusion or transplant setting. Therefore, our literature review for the clinical uses of HLA test results for transfusion or transplantation focused only on studies that provided direct information about HLA matching (considered HLA type, anti-HLA antibodies or both) and clinical outcome.

.Across the entire genome, HLA is recognized as the most important region in relation to disease susceptibility. Many diseases have now been reported to occur more frequently in individuals with particular HLA types. HLA testing can be useful in narrowing diagnostic possibilities and directing future evaluation. For example, the association of HLA-B27 with Ankylosing Spondylitis (AS) was first described in 1973, and remains one of the strongest genetic associations with a common human disease. Therefore, we searched the literature for HLA-B27 and AS, and used the association as an example of the use of HLA testing as an aid for disease diagnosis.

We sought to address the following questions through systematic literature review:

- What is the reported analytical performance of the HLA typing and HLA antibody detection devices?
- How are the HLA test results used to aid donor and recipient matching in transfusion or transplantation, or to aid disease diagnosis?
- What are the risks associated with HLA devices?

1.1. Literature search for analytical performance of HLA devices and their use to aid donor recipient matching

We searched electronic databases PubMed and EMBASE using the following string of keywords for all available articles published before May 1, 2017:

(HLA typing OR HLA antibody detection) AND (“test” OR “assay” OR “method”) AND (transfusion OR transplantation) AND (risk OR safety OR effectiveness OR “survival” OR adverse OR “false negative” OR “false positive” OR incorrect OR mistyped OR invalid OR defect OR accurate OR agreement OR concordance OR discordant OR “correlated” OR pitfalls OR “consistency” OR “consistent” OR ambiguity OR ambiguous OR error OR misassignments OR (clinical outcome) OR TRALI OR GVHD)

The initial search yielded more than 5,000 results. Currently, the most commonly used HLA devices are the solid phase antibody detection kits (ELISA or bead based)

and DNA-based molecular typing kits. The first solid phase HLA antibody detection kit (ELISA-based) was cleared by FDA in 1994, and the first DNA-based HLA typing kit was cleared in 1995. Therefore, we limited our literature search to full text articles published since 1994. After applying these criteria, we generated 1,525 publications excluding duplicates from the two databases.

Titles and abstracts were reviewed to exclude studies not related to the analytical performance or the clinical uses of the HLA assays. We focused on articles that evaluated FDA-cleared HLA devices, and conducted in either the US or in other countries. Most articles do not refer to HLA devices as "FDA-cleared". To determine whether a study used FDA-cleared assay(s), we compared HLA assays' names, type and manufacturer information provided in each paper with our database of FDA-cleared HLA devices. Some studies regarding HLA typing were excluded as they did not indicate the use of an FDA-cleared test (Figure 1). Quantitative HLA antibody detection assays, which are not cleared by FDA, or HLA assays used for post-transplant monitoring, were excluded.

We considered our proposed Intended Use of the HLA devices in determining whether to include or exclude an article:

- HLA typing device: To be used to determine (indicate HLA locus, loci or antigens) to aid donor and recipient matching in transfusion or transplantation.
- HLA antibody detection device: To be used to detect antibodies to (list of HLA antigens) to aid donor and recipient matching in transfusion or transplantation.

A total of 986 articles were excluded during the initial screening for the following reasons:

- Animal study
- Not in English
- Not specific to an FDA-cleared HLA device
- Inadequate analytical performance or clinical use information
- Clinical uses of the HLA device not related to donor and recipient matching in transfusion or transplantation

After reviewing the titles and abstracts, full-texts of the remaining 539 articles were further examined for eligibility. A total of 402 articles were excluded during the full text review using the same criteria listed above.

A total of 137 articles were included for systematic review. Additional information regarding the methodology for inclusion and exclusion criteria are in Figure 1.

1.2. Literature search for the use of HLA-B27 results to aid disease diagnosis in AS

We searched the electronic databases PubMed and EMBASE using the following terms on May 1, 2017 for all available full text articles published in the previous six and half years:

HLA-B27 AND ankylosing spondylitis

The search yielded 786 publications excluding duplicates between the two databases. Titles and abstracts were reviewed to exclude studies for the following reasons:

- Animal or in vitro study not related to AS diagnosis
- Not in English
- No clinical uses information of HLA-B27 in AS
- The clinical use of HLA-B27 not related to "aid disease diagnosis in AS"

A total of 516 articles were excluded following the initial screen. Full-texts of the remaining 270 articles were further examined for eligibility. 169 articles were excluded during the full text review using the similar criteria mentioned above. The remaining 101 articles were included for systematic review. Additional information regarding the methodology for inclusion and exclusion criteria are in Figure 2.

2. Results

The results of our literature review for HLA device analytical performance and test results used for transfusion or transplantation consisted of HLA typing related studies (n=28), HLA antibody detection related studies (n=102), and studies that used both HLA typing and HLA antibody detection devices (n=7). The results of our literature review to evaluate the use of HLA-B27 results to aid diagnosis for AS yielded 101 studies.

3. Analytical Performance of FDA-cleared HLA Devices

In this section, we summarized the articles that have evaluated the analytical performance of FDA-cleared HLA devices; the test results were not used to make clinical decisions in these articles. These studies provided risks to consider if the HLA testing results are used to aid donor and recipient matching in transplantation and transfusion.

3.1. HLA typing devices

HLA typing methods have evolved significantly since the discovery of the first HLA antigen. Originally serological methods were employed to determine HLA types. However, DNA-based molecular assays are now the predominant methods used in many laboratories.

Molecular versus serological HLA typing: We identified several studies suggesting that molecular assays have superior performance compared to serological methods. In one study, 21% to 38% of HLA-A, B and DR antigens were poorly defined by serological typing. These antigens were resolved by molecular typing using sequence specific primer (SSP) kits from One Lambda [1]. Similarly in other studies, FDA-cleared molecular HLA typing kits provided results with higher resolution and/or revealed errors of HLA serological typing [2-6]. In two studies, molecular testing revealed that FDA-cleared serological typing tests generated discrepant results or no results in about 23 – 24 % of the cases [7,8]. Another advantage of molecular typing

is the use of DNA extracted from different tissue types such as buccal swab or blood [9].

Technical limitations of the HLA molecular typing methods: Due to the extreme polymorphism of HLA loci, an HLA molecular kit often uses a large number of primers and/or probes to target different alleles. However, the frequencies of most HLA alleles are low, and genomic DNA samples carrying certain rare alleles are difficult to obtain. HLA kit manufacturers may not have a complete sample panel to validate all primers/probes in a kit. During the use of the kit, the hybridization or amplification patterns from a sample with a rare HLA allele may not completely match the expected patterns on the then most recent HLA sequence database available when the kits were released. HLA sequence databases are frequently updated with new HLA alleles. Rahal et al. identified several new HLA alleles when samples with apparent false-negative or false-positive signals from LABType sequence specific oligonucleotide (SSO) kits (One Lambda) were retyped by other methods [10]. Based on 1200 HLA-DRB1 typing tests, the authors further estimated that probes with fluorescence intensity close to the cut-off value leading to a questionable assignment represented <2% of the assays [10].

The extreme polymorphism of HLA loci presents another major challenge to most HLA molecular typing assays; typing ambiguities. In a review article, Gabriel et al. suggested that allele ambiguities may occur in 41% of HLA-A and 21% of HLA-B typing results [11]. Some of these ambiguities may reflect null alleles in which the HLA molecule is not expressed or truncated, and therefore may generate an alloreactive response. Testi et al. used 146 samples to evaluate the accuracy, sensitivity and performance of a high definition DRB1 kit (LABType SSO DRB1 HD, One Lambda). Twenty-nine samples (20.4%) gave an ambiguous typing with a combination of two or more alleles. Thirty-one samples (21.7%) required cut-off adjustments to one or two beads. With a new kit lot, six beads required a cut-off change [12]. Ambiguous typing results were also reported when using Dynal RELI HLA-DRB SSO kit [13]. Roh et al. reported full concordance typing results without ambiguities when 100 samples were typed by an SSO kit (LIFECODES, Immucor) for HLA-DQB1 locus, which has less allelic polymorphism than HLA-DRB1 locus [14].

Loss of heterozygosity (LOH) has been reported to cause false HLA homozygous typing results in pre-transplant patients suffering from hematological malignancies, who in fact are HLA heterozygous. Linjama et al. reported conflicting HLA assignment by three different typing methods due to LOH in the HLA region [15]. Their data suggest a frequency of 0.4% for LOH in HLA in Hematopoietic Stem Cell Transplant (HSCT) patients. They found that SSP assays (Micro SSP Generic HLA Class I/II DNA Typing Trays from One Lambda and Olerup SSP kit from Olerup) were the most sensitive method for detecting the lost haplotype, followed by SSO (LABType SSO), while Sequencing-Based Typing (SBT) was the least sensitive technique [15]. HLA-mismatched unrelated donor HSCTs are known to carry a significantly increased risk of severe graft-versus-host disease (GVHD) and mortality.

Our literature review did not yield articles examining cross-reactivity or interference of FDA-cleared HLA typing devices.

3.2. HLA antibody detection devices

The first standard assay for the detection of HLA antibodies was the complement-dependent cytotoxicity (CDC) assay. Other methods were later developed including anti-human globulin (AHG)-enhanced CDC assay, flow cytometry crossmatch (FCXM), and the most recent solid-phase assays (SPA) (ELISA, flow cytometry beads-based or Luminex® beads-based assays). The performance of these assays have been extensively studied in the literature.

3.2.1. Luminex®-based assays

Luminex® versus other technologies: Several articles described Luminex® technology as more sensitive than other assays for detecting anti-HLA antibodies [16-19]. Studies have reported that Luminex® assays can detect significantly more HLA antibodies than CDC assays [20-23]. After eliminating immunoglobulin M (IgM), a direct comparison of LABScreen (a Luminex®-based assay from One Lambda) and CDC for the detection of HLA Class I IgG antibodies alone revealed an agreement of about 91% [24]. Kurtulmus et al. reported that Luminex® PRA (formally Panel Reactive Antibody) (Lifecodes) detected more HLA antibodies than flow cytometry assay FlowPRA (One Lambda) [25]. Luminex® assays also showed higher sensitivity than ELISA in several reports [26,27]..

Testing results from Luminex®-based assays have been used to predict crossmatch results (virtual crossmatch (VXM)), and donors having the corresponding HLA antigen(s) can be filtered out [28,29]. One study reported that LABScreen Single Antigen Beads (SAB) correctly predicted the results of CDC and flow cytometric crossmatches in 82.6% and 47.9% of cases, respectively [30]. When another Luminex® assay (Lifecodes) was tested for the prediction of FCXM, a correct prediction rate of 93% was reported [23].

Clinically irrelevant antibodies detected by the Luminex® assays: Several studies described that the distinction between antibodies reactive with intact or denatured HLA cannot be made using regular Luminex-based SAB, as the beads carry a mixture of both antigens. Denatured HLA class I is defined as the HLA α-chain from which β2-microglobulin and peptide within the groove is removed [31]. This causes cryptic epitopes to be exposed, allowing antibody binding to these epitopes that are normally inaccessible on cells [32]. In two studies, sera from patients who had tested positive for anti-HLA antibody using standard SAB were retested. Antibodies to denatured HLA Class I antigens were found in approximately 21% [33] and 39% [34] of patients respectively. Another study reported that 6.2% of Class I specificities recognized by standard SAB assay were defined as to denatured antigens [35]. Antibodies to denatured antigens usually do not fix complement nor produce the same positive FCXM as the anti-native HLA antibodies [33,34,36]. It

has been suggested that preformed antibodies to denatured HLA Class I were clinically irrelevant in kidney transplantation [32,37]. Grenzi et al. observed positive reactions to epitopes presented in denatured forms of HLA-Class II antigens in 1% of patients on the kidney transplant waiting list. The occurrence of this reactivity pattern was found to be associated with female gender and systemic lupus erythematosus (SLE) [38]. Polyreactive antibodies contribute to overall HLA antibody activity detected by Luminex® in some serum, which may explain the conflicting results from using different techniques to detect HLA antibodies, for example, the additional reactivity picked up by Luminex® but not by CDC [39]. HLA antibodies, especially donor-specific antibodies (DSA), are often considered a contraindication for transplantation. However, considering clinically irrelevant antibodies may result in the inappropriate denial of an organ transplantation [34,36] (See Section 4.3 for more information).

Prozone Effect and Interference: Some reports indicated that routine analysis using Luminex®-based SAB assays on undiluted sera can result in an incomplete assessment of HLA antibody levels. Kosmoliaptsis et al. observed increased IgG binding on dilution with the sera from several highly sensitized patients awaiting renal transplantation. This suggests the presence of a prozone effect and misleadingly low antibody binding levels using neat serum alone [40]. Another study reported that prozone effect was more evident in sera from highly sensitized patients, and was only observed using SAB assays [41]. Further investigation showed that treatment with dithiothreitol (DTT) [42,43] or EDTA [43-45] restored maximum IgG antibody binding levels. Similar as DTT treatment, hypotonic dialysis (HD), which also eliminates interference by IgM, improved HLA antibody specificity identification, the determination of DSA strength, and crossmatch predictability compared with values in untreated sera (n = 42) [46]. In this study changes with detected HLA antibody specificity occurred in 61% of HD-treated and 50% of DTT-treated sera. Visentin et al. confirmed that IgM anti-HLA antibodies can interfere with IgG anti-HLA antibody detection in a concentration-dependent manner (LABScreen Mixed, LABScreen Single Antigen) [43]. Gloor et al. reported that antithymocyte globulin (ATG), which is widely used in kidney transplantation, affected Luminex® assay LABSceen PRA by producing positive HLA antibody results [47]. Increased non-specific binding to negative control beads (LABScreen assay) was reported in patients with underlying liver disease and with specific HLA types, which can potentially lead to false negative result [48].

Assay reproducibility: Varied HLA antibody positive rates and mean fluorescence intensity (MFI) values have been reported for the same sample set (n = 20) tested with different Luminex®-based kits in seven laboratories. In this study, antibody assignment agreement ranged from 86% to 93% between kits from two manufacturers (One Lambda and Gen-Probe) [49]. Lot-to-lot variability was observed when testing five lots of LABScreen Mixed assay [50], and three lots of LABScreen SAB [51]. In another study Gandhi et al. reported a better assignment agreement when using the same reagent lot (LABScreen SAB) to detect ant-HLA antibodies in ten samples at four laboratories: the overall concordance for Class-I

was 97% between laboratories and 98% within laboratory at all cutoffs; for Class-II, overall concordance between and within laboratory was 98%. The authors additionally tested the same samples on four consecutive days in one laboratory. The overall concordance was about 98% [52]. A newer LABScreen lot showed more uniform HLA antigen density across all beads than an early lot [53]. The inter-assay and inter-machine mean absolute relative differences (MFI values) of the Lifecodes Lifescreen Delux assay were reported as 12% and 13%, respectively [54].

3.2.2. FlowPRA assay

In the FlowPRA assay the purified HLA class I or class II molecules were immobilized on the surface of beads that are analyzed by conventional flow cytometry. Kao et al. used both FlowPRA and standard CDC to test 102 (for anti-HLA class I) and 45 sera (for anti-HLA class II) from organ transplant patients. The FlowPRA assays showed moderate concordance for anti-HLA class I (k=0.448) and better concordance for class II antibodies (k=0.801), when compared to CDC. FlowPRA assays detected anti-HLA class I not detected by CDC [55].

Our literature review did not identify articles examining cross-reactivity, interference or reproducibility of the FlowPRA assay.

3.2.3. ELISA assays

ELISA assay versus other assays: ELISA assays have been reported in literature to be more sensitive than CDC assays [56,57]. Worthington et al. showed that the concordance between PRA-STAT (an ELISA assay from SangStat) and CDC for the detection of HLA class I-specific antibodies is 87.8% (259 of 295 samples). The authors suggested that PRA-STAT could detect HLA-specific IgG antibodies relevant to transplant outcome that were not detected by CDC [58]. As PRA-STAT detects only IgG antibodies, the agreement between PRA-STAT and CDC results for samples treated with DTT, which leads to the reduction of IgM antibodies, was higher than the agreement between PRA-STAT and CDC without DTT treatment [59]. In another study the concordance between QuikScreen (an ELISA assay from GTI) and AHG-CDC+DTT for IgG antibody detection was reported as 96.2% [57]. When QuikScreen were compared to CDC assay using Antigen Tray from One Lambda, 73 sera were positive only by QuikScreen, 48 sera by both QuikScreen and CDC methods and 16 sera only by CDC method [60]. Gebel et al. described that up to 25% of potential solid organ allograft recipients who would have been reported as non-sensitized based on CDC assays are sensitized when assessed by QuikScreen or FlowPRA [61]. In another study the reported overall concordance was 82.8% between QuikScreen and FlowPRA for the detection of HLA class I antibodies [62]. Although many studies found that ELISA assays are more sensitive than CDC, an earlier study reported eight (8) false-negative reactions in 40 sera tested with PRA-STAT as compared to CDC assay [63]. Harmer et al. have shown that PRA-STAT is less sensitive than flow cytometry [64]. Significantly higher rate of HLA antibody positivity was observed among group O donors than donors from other ABO groups when tested by ELISA (DonorScreen, Immucor GTI

Diagnostics), which was not observed using the Luminex-based assay (LABScreen) [65].

ELISA-based crossmatching assays: Antibody Monitoring System (AMS) is an ELISA-based crossmatching assay developed by GTI Diagnostics. Schlaf et al. reported that in patients characterized by doubtful CDC crossmatches (n = 125), only 18% of these exhibited a positive antibody reaction using the AMS-ELISA. [66]. Altermann et al. reported t that AMS is more sensitive than CDC crossmatch [67]. The concordance between AMS and FCXM assays was 89.7% when performed on 107 sera in which 34 sera contain donor specific antibody (DSA) [68]. In another study when 32 sera with high PRA (>50%) were tested by both AMS and FCXM, discordant results were reported on five samples [69]. Results from another ELISA-based crossmatching assay CROSS-STAT (SangStat) showed an agreement of 94% when compared with consensus CDC results (n = 74) [70].

Concordance between different ELISA assays: The concordance between two ELISA assays (QuikScreen and DynaChip) was reported as 88.2% for class I antibodies and 91.3% for class II antibodies [23]. This study also reported 90.7% concordance comparing B-Screen (an ELISA assay from GTI) and FlowPRA for the detection of HLA class II antibodies. B-Screen failed to detect antibodies to HLA-DQ in a number of samples [62]. Another study reported LAT (formally Lambda Antigen Trays from One Lambda) as more sensitive than two other ELISA methods PRA-STAT and QuikScreen [56].

False-positive ELISA results have been recorded in one study [71]. Thirty three (33) samples from blood donors that were tested positive for HLA class I or II antibodies by DonorScreen (an ELISA assay from GTI) were retested by LABScreen. The investigators noted false-positive reactions for the HLA class I antibody in two donors and for the HLA class II antibody in 19 donors using the DonorScreen method. There were no reported transfusion reactions in the recipients of packed RBCs or platelets obtained from donors who tested positive by DonorScreen method [71].

Our literature review did not find articles examining cross-reactivity, interference or reproducibility of the FDA-cleared ELISA assays.

4. Clinical Uses of HLA Testing Results to Aid Donor and Recipient Matching in Transplantation or Transfusion

HLA devices have been widely used to generate results to aid donor and recipient matching in transplantation and transfusion. Many articles identified by our literature review are retrospective studies investigating the association of HLA matching strategies and clinical outcome. FDA-cleared HLA devices were used in these studies to provide testing results.

4.1. Clinical uses of HLA typing results for donor and recipient matching

HLA matching is important in selection of suitable donors, which is considered as a priority for kidney transplantation and HSCT. The benefit of HLA matching for transplantation of other solid organs has often been debated.

4.1.1. Kidney transplantation

With potent immunosuppression and improved patient management, renal graft survival rates increased immensely during the last three decades. In spite of these improvements, the effect of HLA matching on kidney transplants from both deceased donors and living donors remains significant. Pourfarziani et al. reported that three-year graft survival was significantly better for the recipients with better HLA matches. SSO typing kits from Dynal were used in their study [72]. Similar conclusion was drawn in another study in which DRB1 and DQB1 SSP assays from Olerup were utilized to determine HLA types (n = 1934 patients) [73]. The authors also used the delayed type hypersensitivity (DTH) assay to assign kidney recipients (n = 71) to immune regulator, nonregulator, and sensitized categories. Within the cadaver population, two HLA-DR-matched recipients had a higher frequency of regulated anti-donor DTH than one and zero HLA-DR-matched recipients. Matching for HLA-DR, total class II (DR and DQ), and total HLA (A, B, DR, and DQ) were all found to be statistically significant factors influencing the pattern of DTH regulation [73]. HLA-DR mismatching (HLA-DR typed by Micro SSP HLA Class II DNA Typing Kit from One Lambda) was associated with acute graft rejection [74].

HLA typing kits have been used together with HLA antibody detection assays to identify DSA in transplant recipients. Caro-Oleas et al. reported the use of AllSet Gold SSP kits from Invitrogen for the detection of DSA in 982 patients who received renal grafts from deceased donors after a negative CDC crossmatch. Kidney transplantation outcome was worse among recipients with DSA [75]. In presensitized patients with preformed HLA antibodies, the compatibility for HLA-DP and HLA-C alleles (typed by Olerup SSP kits) was reported as affecting kidney graft survival [76].

4.1.2. Non-renal solid organ transplantation

The value of HLA matching in non-renal transplantation is not as clear as in kidney transplantation. Muro et al. used SSP assays from Dynal and One Lambda and traditional CDC assays for HLA typing of 224 recipient-cadaveric liver donor pairs. HLA-A and DPB1 matchings were found as independent risk factors for graft loss [77]. Castillo-Rama et al. analyzed a single-center cohort comprising of 896 liver transplants. HLA-A, HLA-B, and HLA-DR were typed using typing trays from One Lambda in CDC assays. No significant beneficial or detrimental effects associating the degree of HLA donor and recipient matching and liver graft survival have been observed [20]. Similarly, there was no association found between HLA mismatch and acute rejection or graft survival in liver transplantation in which HLA-A, HLA-B and HLA-DR were typed using SSO kits (Lifecodes) [78].

HLA-typing trays from One Lambda were used together with anti-HLA sera from a local laboratory to evaluate the influence of HLA mismatches on lung transplantation outcome, in which obliterative bronchiolitis (OB) was the principal cause of chronic allograft loss. The authors identified mismatching at HLA-A locus as a significant risk factor for OB, and noted that HLA-DR mismatches indirectly influence the development of OB [79].

4.1.3. Hematopoietic stem cell transplantation (HSCT)

Many studies have shown that matches at the HLA-A, HLA-B, HLA-C, HLA–DRB1 and HLA–DQB1 loci (10/10 matched) improve survival and the overall HSCT outcome. Matching for HLA class I and II alleles (detected by Dynal SSP kit) was found to be associated with low incidence of severe acute GVHD in HSCT recipients [80,81]. Huo et al. reported that HLA-B mismatch (determined by Monoclonal Tray and Micro SSP Typing Tray from One Lambda) is associated with significant adverse effects on acute GVHD and transplant-related mortality, reduced overall and leukemia-free survival [82]. Using data from a combination of FDA-cleared kits (SSP kits from Dynal and One Lambda) and for research use only kits, 57 bone marrow donor/recipient transplant pairs were fully matched for the alleles HLA-A, B, C, DRB1 and DQB1 and also for the alleles DRB3, 4 and 5 in order to eliminate the alloimmune impact of these HLA molecules. In this setting, the authors found that two HLA-DPB1 incompatibilities are associated with severe acute GVHD and reduced patient survival [83]. Similarly, a distinct trend to poorer overall survival was reported with double HLA-DPB1 mismatch (Lifecodes SSO kit) [84]. However, Pan et al. reported mismatches at HLA-DP loci did not have major effect on outcomes [85].

High resolution HLA molecular typing is recommended for HSCT. Studies found that previously matched recipient-donor pairs are sometimes determined to be mismatched when high resolution molecular techniques are used. Kassar et al. used Dynal SSP typing kits and identified significantly more (36 vs. 8) mismatched pairs than the previously used low resolution assays in 69 bone marrow transplant procedures [81]. Sun et al. identified additional four (4) mismatches with a higher resolution molecular assay in 37 patients awaiting HSCT and related potential donor combinations that were previously typed by a low resolution SSP kit from Pel-Freez [86]. They concluded that some HLA identical donor and recipient pairs assigned by low resolution typing methods were mismatched at their allelic levels, which might lead to severe GVHD and poor outcome after HSCT.

In recent years, umbilical cord blood has been used successfully as an alternative haematopoietic stem cell source to treat a variety of hematologic, immunologic, genetic, and oncologic disorders. Studies have shown that some degree of HLA mismatches is acceptable when using cord blood. In one study the engraftment rate and GVHD after cord blood transplantation did not significantly correlate with extended HLA matching including HLA-C and HLA-DQB1 (typed by SSP HLA typing kit from Dynal) [87].

4.1.4. Transfusion

HLA antigens are associated with several complications of blood transfusion. Although HLA typing assays have been used in many studies investigating the clinical relevance of the HLA system in blood transfusion, only a few of the published articles included the name of the HLA typing assays used.

Xia et al. used AllSet Gold SSP (Invitrogen) to select HLA matched platelets for 23 patients with platelet transfusion refractoriness. Significantly higher platelet recovery values were obtained with HLA-matched platelets in comparison with random platelets [88].

4.2. Clinical uses of HLA antibody detection results for donor recipient matching

Transplant or transfusion recipients or donors who have had sensitizing events develop antibodies against HLA antigens. In general the presence of pre-transplant HLA antibodies (also called preformed HLA antibodies), and specifically to those HLA antigens that are expressed by the organ donor (Donor Specific Antibodies - DSA), represents the dominant reason for hyperacute and acute rejection of renal allografts and allografts of other solid organs. They are thus regarded as contraindication for grafting according to the transplantation guidelines of most countries and international societies [66,89].

4.2.1. Kidney transplantation

HLA antibodies detected by Luminex®-based assays: It appears that early reports about the value of DSA detection using Luminex®-based assays in kidney transplantation presented more controversial views, whereas later studies provided more support [90]. Evidence provided by several studies show that Luminex® assays can identify clinically relevant HLA antibodies including the ones missed by CDC assays. In a study involving 892 patients who received a graft from deceased donors after a negative CDC crossmatch, an SAB assay (Tepnel Lifecodes) was used to detect anti-HLA DSA. Graft survival was significantly worse among 103 patients with anti-HLA DSA compared to both patients with non-DSA anti-HLA and patients without HLA antibodies [75]. Similar findings were reported with AHG-CDC crossmatch negative recipients who were retested using Luminex PRA assays from Tepnel Lifecodes [91,92].

Higgins et al. reported that rejection occurred significantly more in DSA positive patients in the first year compared with the DSA negative patients (Luminex SAB assay, One Lambda) [93]. Anti-HLA DSA (LABScreen) was associated with higher incidence of an episode of rejection in the first 30 days, and with death-censored graft failure [94]. Higher HLA-mismatch numbers were observed in kidney transplant recipients with anti-HLA-DSA (Tepnel Lifecodes) [95]. HLA-Cw antigen has been considered to be less immunogenic than other HLA antigens such as HLA-A or HLA-B. Ling et al. reported 100% survival in eight (8) kidney

transplantation recipients with anti-HLA-Cw DSA (determined by LABScreen SAB) [96].

Colombo et al. found that using LABScreen method, 18% of patients on the kidney waiting list were considered and managed as sensitized as compared to 7% when testing with CDC alone [24]. Bostock et al. determined that the probability of receiving a deceased donor kidney transplant is inversely related to the % PRA, although a higher risk for not receiving a kidney transplant becomes evident with a PRA >20% (LABScreen) [97]. Pregnancy is a strong sensitizing event (HLA antibody detected by LABScreen assays) in women awaiting kidney transplantation [98,99]. However, some Luminex® (Lifecodes Lifescreen Deluxe) positive patients did not experience any immunizing events [42].

HLA antibodies detected by FlowPRA: Renal allograft recipients with pretransplant HLA antibodies detected exclusively by the FlowPRA assay exhibited a decreased mean time to first rejection episode in comparison with FlowPRA-negative recipients [23]. Ishida et al. described FlowPRA as more sensitive than FCXM, and FlowPRA positive recipients showed more humoral rejections [100].

HLA antibodies detected by ELISA-based assays: Li et al. used LAT assays to detect HLA antibodies in a study including 1297 renal transplant recipients. The incidence of delayed graft function was significantly higher in recipients with HLA antibodies than recipients without HLA antibodies [101]. In another study the recipients with HLA antibodies (detected by LAT) had a worse two year outcome [102]. ELISA assay PRA-STAT also detected HLA antibodies relevant to renal transplantation outcome [58,103]. A positive signal in the AMS-ELISA crossmatch has shown a “high degree” of concordance (n = 7 out of 9) between the loss of kidney function due to clinically proven rejection episodes, while only one of the patients negative for DSA using the AMS-ELISA (n = 24) suffered from a biopsy-proven rejection [23]. A few studies reported that HLA antibodies as detected by ELISA assays (LAT from One Lambda) had no significant impact on graft survival [101,104]. DSA was not specifically examined in these studies.

4.2.2. Heart transplantation

DSA detected by Luminex®-based assays: Raess et al. reported that the presence of pretransplant DSA (detected by LABScreen SAB assay) is predictive for short-term but not long-term survival after heart transplantation. As compared to CDC PRA, LABScreen determined DSA class I predicted a decreased short-term survival with a four times higher sensitivity. HLA antibody screening with LABScreen Mixed did not predict survival [105]. In another study DSA in pretransplant sera from 85 heart transplant recipients with negative CDC crossmatch was tested by LABScreen SAB assay, and showed 90% accuracy when compared to FCXM results. Recipients with pretransplant DSA and positive FCXM have significantly higher rates of antibody-mediated rejection (AMR) and cell-mediated rejection (CMR) compared to recipients with negative DSA or FCXM [106]. A study with 264 adult heart transplant recipients also reported an association of preformed DSA (LABScreen

Mixed and Single Antigen) with pathogenic AMR [107]. In heart transplant patients, logistic reasons limit performing a prospective crossmatch. SAB defined DSA can be potentially used as a surrogate for FCXM. Zangwill et al. examined the effect of the virtual crossmatch (VXM) on wait times and outcomes. HLA antibody specificities were determined by Luminex® assay from One Lambda. The VXM was concordant with the retrospective crossmatch in eight out of nine cases [108]. The authors suggested that the use of a VXM can lead to shorter wait times and better outcomes as a listing strategy for sensitized children requiring cardiac transplantation.

4.2.3. Liver transplantation

Luminex® assay (LABScreen) was used in a study in which preformed anti-HLA antibodies were detected in 20.2% of 810 liver transplant recipients. These antibodies were associated with shorter graft survival within the first year post-transplant. Specifically the study found that Luminex-detected anti-HLA class II antibodies played a significant role in decreasing graft survival [20]. Wozniak et al. evaluated six pediatric ABO-compatible liver transplant recipients diagnosed with early acute AMR. Three of four patients with available pretransplant HLA antibody data (LABScreen and FlowRPA) were positive for HLA antibodies [109]. However, in another study (n = 97), no association was found between graft dysfunction at 60 months and positive Luminex® (Lifecodes) PRA values [22]. In addition to Luminex® assay, the presence of HLA antibodies detected with CDC crossmatch suggested a deleterious effect on liver transplant outcome, and was associated with an increased incidence of early graft loss and rejection episodes [110].

4.2.4. Lung transplantation

Smith et al. used Luminex® assays (Lifecodes and LABScreen) to detect pre-transplant DSA in 425 adult lung transplant recipients. Patients with DSA (n = 27) had an actuarial 1-year survival of 51.9% compared with 77.8% for those with non-DSA anti-HLA (n = 36), and 71.8% for patients with no detectable anti-HLA (n = 362). Graft failure in the DSA group occurred early after transplantation [111]. Girnita et al. reported that HLA antibodies (determined by LAT ELISA) are significantly associated with more persistent-recurrent acute rejection and high-grade acute rejection episodes [112].

The role of anti-HLA Class I versus Class II DSA in lung transplant has not been well defined. Smith et al. revealed that Class II DSA had significantly poorer survival, but to a lesser extent than Class I DSA [111]. Brugiere et al. reported that freedom from bronchiolitis obliterans syndrome was lower, and mortality was higher for patients with preformed Luminex®-detected class II DSA, but did not differ for patients with and without class I DSA [113].

4.2.5. Intestinal transplantation

Ruiz et al. reported the presence of pretransplant DSA (FlowPRA Single Antigen, One Lambda) in an intestinal transplantation recipient who developed immediate antibody-mediated rejection [114].

4.2.6. Hematopoietic stem cell transplantation (HSCT)

Most of the patients who need HSCT do not have a matched related donor. For these patients, a matched unrelated donor (MUD) transplantation may be preferred. The presence of HLA antibodies (detected by LABScreen) in MUD HSCT recipients was reported as a risk factor for GVHD and overall survival [85]. DSA were associated with primary graft failure [115]. HLA typing is generally performed for HLA-A, HLA-B, HLA-C, HLA-DQB1 and HLA-DRB1 loci for HSCT matching. To examine the effect of HLA-DPB1 mismatch on clinical outcome, LABScreen assay was used in a study to prospectively determine DSAs in 592 MUD transplantation recipients. All DSAs were directed against the HLA-DPB1 molecule in these MUD recipients. In multivariate analysis, the authors identified DSA to DPB1 as the only factor highly associated with graft failure [116]. Cord blood is a valid alternative to bone marrow and peripheral blood stem cells in HSCT. Studies suggest avoiding HLA mismatches at loci in which patients have preformed HLA antibodies. Takanashi et al. identified 20 cases with HLA antibodies (FlowPRA and LABScreen) against cord blood HLA from 386 cord blood transplants. Recipients with such antibodies had significantly lower neutrophil recovery and platelet recovery [117].

In addition, the presence of anti-HLA class II antibodies in unrelated HSCT donors (detected by LABScreen) was associated with a higher cumulative incidence for a first episode of either acute or chronic GVHD. Identifying specificities of the anti-class II antibodies revealed that half of the alloimmunized donors had recipient-specific antibodies (RSA), directed mainly against mismatched HLA-DPB1 alleles. Owing to the lack of linkage disequilibrium between HLA-DPB1 and the rest of the extended HLA haplotype, approximately 86% of 10/10-matched unrelated donors are mismatched at the HLA-DPB1 locus [118].

4.2.7. Pancreas Transplantation

Eby et al. evaluated the use of virtual HLA crossmatching based on DSA data (LABScreen Single Antigen) in pancreas transplantation. Death-censored graft survival, patient survival, and rejection rates were comparable among the recipient groups of VXM and FCXM. The time from organ arrival at the hospital to operation start was significantly shorter in the VXM-only group compared with the VXM + FCXM group. The authors concluded that VXM helps minimize cold ischemia time without increasing rejection or adversely affecting graft survival [119].

4.2.8. Transfusion

Some transfusion reactions are triggered by HLA antibodies present in the patients whereas others are initiated by HLA antibodies or HLA reactive cells present in the transfused products. Lin et al. used LABScreen assay and detected the presence of a recipient-specific antibody significantly greater in the definite and possible

transfusion-related acute lung injury (TRALI) cases compared to other cases [120]. HLA antibody detection devices are used in blood establishments that are adopting TRALI risk reduction strategies. A major challenge of screening donors for HLA antibodies is how to decrease the risk of TRALI for recipients while maintaining an adequate supply of plasma containing blood products. LABScreen assays allow the end users to set cutoff levels for HLA antibody testing. If the cutoffs are too sensitive, donation loss may adversely impact blood availability. In one study (8171 donors enrolled) the relative impact of imposing various screening assay cutoffs or pregnancy stratification was examined in relation to detection of HLA antibody–reactive donations and loss of donors and donations [121]. Samples with a broader array of HLA antibody specificities or higher HLA antibody screening values have higher probability of matching a cognate antigen in a recipient. Depending on the TRALI risk reduction strategy used, the potential loss of donations ranged between 0.9 and 6.0% [121]. In addition to traditional CDC assays, both ELISA (LAT) and Luminex® (LABScreen) have been used to evaluate HLA antibodies in acute non-hemolytic transfusion reactions (ANHTRs). High incidences of HLA antibodies in both patients and donors associated with ANHTRs were observed [122].

HLA antibody detection devices have been used to examine HLA alloimmunization rate after blood transfusion, which may affect donor matching for future transplantation or transfusion. In one study, none of the five aplastic anemia patients who received leukoreduced blood products was positive for HLA antibodies (determined by FlowPRA), compared to six HLA antibody positive patients from a group of 15 patients who had received non-leukoreduced components previously [123]. However, Balasubramaniam et al. reported that transfusion even in the postleukodepletion era continues to pose a risk of sensitization. LABScreen assay detected HLA antibodies in seven of the 42 male patients (16.7%) on kidney waiting list who received at least one unit of leuko-depleted blood. Of the remaining 74 male patients without a history of transfusion, 3 (4.1%) were found to have HLA antibody. In this study a history of blood transfusion gave a relative risk of 4.1 of developing HLA antibody [124].

Patients who are refractory to platelet transfusion because of HLA alloimmunization are generally given HLA-matched or crossmatched platelets. In an observational study with 114 patients, the percentage of platelet recovery was determined in 1621 platelet transfusions. The presence of HLA antibodies in recipients (detected by CDC assay using frozen cell trays from One Lambda) and donors' HLA types were used in the antibody specificity prediction (ASP) method (similar to virtual crossmatch) to predict whether a patient's antibodies will react with donor HLA antigens [125]. The authors compared the platelet recovery of platelets selected by the ASP method with the platelet recovery of those that were HLA matched, crossmatched, or randomly selected. Their conclusion was that the selection of platelets for transfusion to refractory alloimmunized patients based on HLA antibody specificity prediction is as effective as providing HLA-matched or crossmatched platelets, and significantly superior to randomly selected platelets. Far more donors were identified in a pool of HLA-typed donors by the ASP method

than by HLA matching [125]. In another study the PRA determination by Luminex® (LABScreen) or flow cytometry (FlowPRA) assays appeared better correlated than determination by CDC with the ability to find crossmatch-compatible platelets [126].

4.3. Challenges related to the clinical use of HLA antibody detection devices

Published studies indicated that not all antibodies determined by Luminex® assays could be considered clinically relevant in solid organ transplantation. Couzi et al. showed that only the combination of LABScreen SAB and FCXM results, but not SAB alone, identified DSA predicting short-term outcome of sensitized kidney transplant recipients [127]. Interestingly, in another report 118 CDC negative renal transplant recipients with graft loss did not show a higher incidence of DSA (detected by LABScreen) than 118 matched controls without graft loss [128]. Thiyagarajan et al. used LABScreen to determine preformed HLA antibodies without CDC testing. The HLA class I, class II, and DSA-positive groups showed no difference in renal function or graft survival at two years [129]. Other studies similarly showed that the detection of non-DSA HLA antibodies is not associated with kidney (Lifecodes assays from Tepnel) [75] or lung graft outcome (SAB assay from One Lambda) [113].

There was also a report suggesting that not all HLA antibodies detected by Luminex® assays in donors are associated with transfusion reactions [130]. In this study LABScreen assay detected Class I antibodies in 15% plateletpheresis donors and Class II antibodies in 21% donors. However, there were no reported reactions after transfusion of the platelets from these donors (n = 136) in 265 episodes to 67 patients.

Several studies suggested that considering antibodies to cryptic/denatured HLA epitopes for HLA matching could lead to unjustified exclusion of potential donors. One study reported that 11% of the class I DSAs detected by regular SAB analysis were caused by reactivity with denatured HLA [131]. The authors also found that patients with DSA to intact HLA showed a significantly lower graft survival rate compared to those without HLA class I DSA, whereas reactivity to exclusively denatured HLA was not associated with decreased graft survival [131]. Oaks et al. determined that the antibody responses to cryptic/denatured HLA epitopes are not associated with factors commonly thought to be associated with antibody responses to HLA such as age, gender, transfusions or presence of circulatory support [33]. Sicard et al. examined renal transplant recipients who were tested positive for preformed DSA by Luminex® but without history of an immunizing event or positive CDC crossmatches, and observed favorable outcomes of these recipients with DSA of unknown etiology [132]. Several other studies reported similar findings [21,133]. It has been speculated that these HLA antibodies are produced to cross-reactive epitopes found in microorganisms, ingested proteins and allergens—making them natural antibodies. One possible problem is denaturation of the HLA antigens during purification and attachment to solid surface such as beads, exposing cryptic epitopes [133]. Gombos et al. described the finding of antibodies with high-

MFI value in some unimmunized patients with HLA specificities that are rather common in general population [21]. Increased percentage of nonspecific/natural antibody specificities was reported when using lower cutoff values for LABScreen assay [134].

While considering antibodies with no clinical impact for donor and recipient matching would lead to unjustified exclusion of potential donors, a test may give false negative results when a “public” antibody, for which the corresponding epitope is shared by more than one HLA antigen/bead, is ‘spread too thin’ over a copious number of beads [135]. In addition, HLA genes are known to be extremely polymorphic, therefore, it is not currently possible to represent all antigens in a SAB assay [136]. Roberts et al. suggested that laboratories using SABs alone may fail to recognize potentially clinically relevant antibodies [135]. Several groups recommended the use of multiple platforms [26,135], such as using an SAB test together with a Luminex® screening assay [137].

5. HLA-B27 Testing Used to Aid Ankylosing Spondylitis Diagnosis

Ankylosing Spondylitis (AS) is the prototype of immune-mediated inflammatory rheumatic diseases grouped under the term spondyloarthritis (SpA). A wider group of SpA has been proposed by Assessment of Spondyloarthritis International Society (ASAS), and the patients are sub-grouped into (1) a predominantly axial disease, termed axial SpA including AS and non-radiographic axial SpA (nr-axSpA); (2) peripheral SpA [138]. Axial SpA encompasses a spectrum of disease severity that spans from self-limited inflammation to bony destruction of the spine. AS is a well-characterized, chronic and progressive form of axial SpA.

5.1. Association of HLA-B27 with AS

In most populations studied worldwide, HLA-B27 is strongly associated with AS [139-141]. Many studies have shown that the frequency of AS and SpA parallels the frequency of HLA-B27. Cauli et al. reported a higher number of HLA-B27 molecules on the cells of AS patients compared to HLA-B27-matched controls [142,143]. However, in many populations only a small fraction (1~ 5%) of HLA-B27 positive subjects develop the disease [144]. Although HLA-B27 is neither sufficient nor necessary for the occurrence of AS, in the appropriate clinical situation, HLA-B27 may be of value in supporting the diagnosis of AS [138,145-148]. One article estimated both sensitivity and specificity of HLA-B27 for the early diagnosis of AS/axial SpA as 90% in the European Caucasian population [149]. The prevalence of HLA-B27 was reported as higher in AS than in nr-axSpA in several publications [150,151], while other studies found similar prevalence [152,153].

5.2. HLA-B27 prevalence in AS patients from different populations

The strength of the association between HLA-B27 and AS has been extensively studied, which need to be considered when using HLA-B27 results to support AS diagnosis in different populations. The prevalence of HLA-B27 and its association

with AS varies markedly between racial and ethnic groups with a general decline in frequency from north to south across the world [154].

- Rashid et al. reported 39.7% individuals in a Inuit population were HLA-B27 positive, while all patients with AS were HLA-B27 positive [155].
- In a U.S. national survey, the prevalence of HLA-B27 was 7.5 % among non-Hispanic whites while it was lower at 4.6 % in Mexican Americans and approximately 1.1 % among non-Hispanic blacks [156]. While 85–95 % of white AS patients have HLA-B27 [157], the prevalence of HLA-B27 among African American AS patients was reported at 50% [156].
- HLA-B27 was positive in 82.9% Italian AS patients [158], 90% in young Greek males patients [159], 83% in a Europe group with AS, 72.1% in Romanian AS patients [160], 71% in Latin America AI patients [161].
- HLA B-27 was found in about 80% Juvenile ankylosing spondyloarthritis patients from Russia [162].
- HLA-B27 was present in over 90% AS patients from Chinese Han population [163-165], Xinjiang Uygur [166] and Taiwan [167]. It was found that AS patients from southern China had a higher prevalence of HLA-B27 than patients in Northern China [168].
- The incidence of HLA-B27 is as low as 0.4% in Japan. Among 13 Japanese AS patients, HLA-B27 was present in two patients [169].
- The prevalence of HLA-B27 among AS patients is over 70% in Iran [170,171], 62.5% in Vojvodina [172], 76% in India [173], 69% in Saudi Arabia[174], 71% in Jordan, 73% in Qatar[175], 74% in a group of Arabs [176], 58.6% in Egypt [177], 76.9% in New Zealand Māori [178] and 45.6% in Macedonia [179]. Three studies reported HLA-B27 prevalence in Turkey AS patients as 67.5% [180], 73.7% [181] and 90.2% [182], respectively.
- HLA-B27 was observed in 29%, 45% and 64% Moroccan AS patients in different studies [154,183,184], and 61.7% in Tunisian AS patients [185].
- Close to 41% of Colombian patients with spondyloarthritis were HLA-B27 positive of which 52.9% has AS and 42.5% axSpA [186].

Although the worldwide distribution of AS is closely related to the prevalence of HLA‑B27, rare exceptions to this association have been observed. In West Africa, for example, AS remains uncommon despite some ethnic groups having an HLA‑B*2705 prevalence similar to European populations [187-190]. One study revealed that the HLA-B*2705 allele occurred in seven (50%) of the 14 cases of AS from Burkina Faso [191], while another study reported the prevalence of B27 was 4.6% in AS patients from Ghana [192].

5.3. HLA-B27 subtypes/alleles and AS

HLA-B27 has a high degree of genetic polymorphism, with at least 160 known subtypes based on one or more amino acid sequence differences [193,194]. Some of these subtypes exhibit differential association with AS. The most common subtypes associated with AS are HLA-B*27:05 (Caucasians), HLA-B*27:04 (Chinese), and HLAB*27:02 (Mediterranean populations) [193,195]. Although AS cases have been reported as having many other B27 subtypes, for most alleles the number of cases reported is too few to definitely comment on their relative strength of association with the disease [139].

The common white European subtypes, B*2705 and B*2702, are strongly associated with AS [196]. In Northern Norway, the high prevalence of AS is linked to the 15% population prevalence of HLA-B27. HLA-B*2705 was present in 98% and HLA-B*2702 in 2% of AS patients [197]. The almost exclusive presence of HLA-B*2705 in AS patient in this region contrasts with findings in Southern Europe, Asia and Africa, where there is a variable mix of HLA-B*2705 with other subtypes [179]. In contrast to the findings from many studies, meta-analysis of published studies showed a negative association between HLA-B*2705 and susceptibility to AS in the southerners of China [198], or no association worldwide [199].

The primarily Asian subtype HLA-B*2704 is at least as strongly associated with AS as HLA-B*2705 in the same population, with some studies suggesting that it may be more strongly associated [164,165,198-202]. HLA-B*2707, also mainly found in Asians [196], seems equally strongly associated with AS as HLA-B*2705, with one known exception (the Greek Cypriot population) [200]. There is evidence to suggest that HLA-B*2706 (found in Southeast Asian population) and HLA-B*2709 (found in Sardinia and southern Italy) have reduced strength of association with AS [190,194,200,203]. Meta-analysis of seven studies showed a protective effect of HLA-B*2706 on development of AS [198,204].

5.4. HLA-B27 and AS characteristics

Many studies have found HLA-B27 positive patients develop symptoms earlier than their B27-negative counterparts. HLA-B27-positive patients with AS are younger at the onset of disease and diagnosis as compared to patients who are HLA-B27-negative [140,156,174,183,195,205-208]. HLA-B27 appears to lower the threshold for developing the disease at an earlier age [208]. In AS patients, radiographic progression occurred significantly faster in HLA-B27 positive patients [209]. In early axial SpA, HLA-B27 has been shown to be associated with earlier onset of inflammatory back pain (IBP), early diagnosis, axial inflammation (spine and sacroiliac joints), radiographic damage of the sacroiliac joint and lower frequency of psoriasis [145].

HLA-B27-positive patients with AS have greater familial occurrence as compared to patients who are HLA-B27-negative [183,210,211]. The risk of developing AS is much higher for HLA-B27-positive first-degree relatives of HLA-B27-positive AS patients [187,212]. The presence of HLA-B27 was found significantly higher in

familial AS than sporadic ones [213,214]. HLA-B27 was reported as more common in male AS patients than female patients [154,195,210,215,216]. It has been suggested that HLA-B27 is a valuable informative diagnostic tool especially in regard to male patients who have a family history of AS [154].

It has been reported that clinical features and severity of AS is influenced by HLA-B27 status. HLA-B27-positive patients with AS have frequent association with anterior uveitis [183,208,217,218], involvement of lumber spine, thoracic spine and hip joint, and less frequent occurrence of psoriasis and inflammatory bowel disease as compared to their HLA-B27-negative AS peers [145,208]. HLA-B27 positivity in AS was reported to be related with peripheral arthritis [195], though other studies showed no significant relationship [208]. It has been suggested that patients with acute anterior uveitis, especially those who are HLA-B27 positive, should be evaluated for clinical features of SpA [217]. HLA-B27 positive patients have a significantly higher median Bath ankylosing spondylitis disease activity index (BASDAI) [219], and worse sacroiliitis [205] than HLA-B27 negative patients.

Some studies found that homozygosity for HLA-B27 increases disease risk and the likely hood of familial aggregation [140,220] and influences clinical manifestations [139]. However, other studies have examined but did not find a similar relationship [145,194,208,221]. AS patients who carried both HLA-B27 and HLA-B60 showed a high susceptibility to AS [167,222].

The association of specific HLA-B27 subtypes and AS disease features has been evaluated. Although the positive rate of HLA-B27 subtypes in Juvenile-Outset AS (JAS) group had no statistical difference compared with adult-outset AS (AAS) group, clinical features, including peripheral arthritis, enthesitis, BASDAI, erythrocyte sedimentation rate, and C reactive protein were reported as significantly higher in JAS patients with HLA-B∗2704 than those who were B27-negative. HLA-B27-positive men with AS had significantly higher Bath AS Radiological Index lumber score [223]. Enthesitis and ESR were significantly higher in patients with HLA-B∗2705 than those with B27-negative.The onset age of HLA-B∗2715 group was much earlier than other groups [224]. Uveitis was observed more in HLA-B*2704-positive AS patients from India comparing to HLA-B*2705-positive AS patients [225]. However, in a Chinese population, HLA-B*2705-positive patients demonstrated a significant increase in the incidence of uveitis and dactylitis compared with HLA-B*2704-positive patients [202].

5.5. HLA-B27 and patient classification criteria/referral strategies

The majority of research performed over the last two decades has used the modified New York Criteria to identify patients with AS. Of the patients having one or more of the three SpA features, 15% were classified as having AS based on the modified New York Criteria [226]. The modified New York Criteria have potential limitations in clinical practice as they are designed to identify patients with established AS and requires X-ray evidence of sacroiliitis [151]. Attributable mainly to the late appearance of definite sacroiliits on radiographs, the diagnosis of AS may get delayed

6 to 10 years [138,227]. As early diagnosis has now become increasingly important, the ASAS developed criteria for axial SpA with the goal of identifying more patients in the spectrum of inflammatory back pain, including patients with early AS [138,228,229]. The ASAS criteria for axial SpA require patients to have back pain for at least three months and age of onset less than 45 years. Subsequently, patients must meet clinical or imaging criteria. The imaging criteria include evidence of sacroiliitis on magnetic resonance imaging (MRI) or x-ray and at least one additional clinical feature. The clinical criteria (or the HLA-B27 arm) include the presence of HLA-B27 and at least two other SpA features. Both HLA-B27 and sacroiliitis on MRI play a major role in the new ASAS classification criteria [151,227,230]. The sensitivity of the entire set of ASAS criteria for axial SpA was 82.9% and the specificity was 84.4% [228]. The imaging arm showed excellent specificity (97.5%), but it has a low sensitivity (66.2%). The HLA-B27 arm has reasonably good sensitivity and specificity (~80% for both) [138].

Salehi-Abari et al. designed an Iran criteria for early diagnosis of AS. AS is considered as the diagnosis if the patient fulfils one of the followings: (1) six clinical points; (2) five clinical and imaging points; (3) if HLA-B27 is positive, five clinical points or four clinical and imaging points; Sensitivity for this Iran criteria for AS was calculated as 100 % [231]. Jung et al. developed the AS genetic risk scoring model consisting of five genetic components: HLA-B27, three copy number variations, and one single nucleotide polymorphism. This combination was reported as more sensitive and more specific than HLA-B27-only typing for the identification of individuals at high risk for AS [232]. HLA-B27 testing is not considered useful as a single diagnostic test in a case report on one patient with low back pain without further signs or symptoms of spondyloarthropathy [233].

As a small proportion of individuals positive for HLA-B27 develops AS, testing healthy individuals for HLA-B27 was not considered as a useful method for identifying patients with preclinical AS [150]. Several studies evaluated referral strategies for axial SpA patients with chronic back pain (duration > 3 months) and age at onset of back pain (< 45 years). Referral Strategy 1 required the presence of at least one of the following criteria: IBP, HLA-B27 positivity, or sacroiliitis detected by imaging. Referral Strategy 2 required the presence of at least two out of the following five screening criteria: IBP, HLA-B27, sacroiliitis, family history for AS, and good response of the back pain to nonsteroidal anti-inflammatory drugs. In a multicenter study conducted in Germany, HLA-B27 positivity was used as a referral criterion in 44.7% of patients referred by Strategy 1, and 38.0% of the referred patients were diagnosed with AS. When used as a referral criterion in 52.5% of patients referred by Strategy 2, 31.5% of whom were diagnosed with AS [234]. When Strategy 1 was used in a monocenter study in Germany, 46% patients were diagnosed with axSpA if HLA-B27 is the only referral parameter, about half of which were classified as having AS [235]. When those two referral strategies were used in a study on patients from 16 countries, HLA-B27 showed a positive predictive value of 67.1% for axial SpA diagnosis. Most patients diagnosed with axial SpA were classified as AS [236]. It was

noted that referral based on HLA-B27 varied widely by country, likely due to the limited access to HLA-B27 testing in some referral centers [236].

5.6. HLA-B27 and diagnosis delay

Delayed diagnosis (DD) has been reported with worse outcomes and unfavorable treatment responses in patients with axial SpA [237]. The lower DD in Italian female AS patients in comparison to that in male was likely related to higher HLA-B27 positivity in female [158]. DD of AS in HLA-B27-negative patients was reported significantly longer than HLA-B27-positive patients [170,238]. Positive HLA-B27 may lead to more progressive disease and subsequently, earlier diagnosis. In addition, positive HLA-B27 may be a reason for evaluating patients more precisely, leading to earlier diagnosis [238].

6. Overall Literature Review Conclusions

6.1. Analytical performance of the HLA typing and HLA antibody detection devices

Our literature review included studies that evaluated the analytical performance of FDA-cleared HLA devices used in the setting of transplantation and transfusion. These studies provide important information regarding assay specificity, reproducibility, comparability and potential interference for HLA antibody detection devices.

Overall, the studies showed that these devices performed comparably with some showing advantage over others in terms of sensitivity and specificity. Some factors, such as denatured HLA antigens presented on the beads, prozone effect and interferences, may affect HLA antibody detection. Because of the widespread polymorphism of the HLA system, test results may be variable. In addition, HLA typing ambiguities remains as a major challenge with many devices. Apparent false negative or false positive HLA molecular typing results may need further investigation to rule out possible new alleles.

6.2. Clinical use of HLA devices in transfusion, transplantation and disease diagnosis

HLA assays have been widely used to aid donor and recipient matching in transfusion and transplantation. The studies generally revealed that HLA assays are important in matching of donors and patients, mitigating transfusion reactions such as TRALI, and for the diagnosis of diseases such as Ankylosing Spondylitis. We identified relatively few studies evaluating the use of FDA-cleared HLA typing devices in clinical settings. This may be due in part to the fact that the practice of HLA antigen/molecular typing is widely accepted and has been utilized for many years. While it is recognized that other factors can influence clinical outcome, these studies showed that HLA mismatch negatively impacted patient outcome. Many other studies examined the association of preformed HLA antibodies and clinical outcome. In general, the presence of HLA antibodies in patients or donors, especially DSA, is associated with worse clinical outcomes. In addition, identifying and considering

HLA antibodies with no clinical impact may lead to unjustified exclusion of potential donors.

Due to the role of immune system in many diseases, certain HLA types are associated with an increased risk for disease. The association of HLA-B27 and AS provides a good example of the use of HLA test results to support disease diagnosis. Incorrect HLA-B27 test results may affect or delay AS diagnosis.

6.3. Risks associated with HLA devices

While studies showed acceptable analytical performance of HLA devices and widespread clinical use, several device limitations identified may cause false positive or false negative HLA test results. Such results could lead to adverse patient outcomes, poor graft survival, transfusion reactions, or incorrect or delayed diagnosis of related conditions.

6.4. Discussions of Strengths and Limitations

There were notable strengths in the papers reviewed. Many studies assessed the analytical performance of HLA devices and provided information on potential limitations. There were multiple studies that examined the use of HLA devices in select clinical populations and its usefulness as an aid to disease diagnosis and donor matching.

In terms of limitations, we were unable to include many articles in the literature review because it was unclear whether FDA cleared devices were used in the studies.

6.5. Conclusions

Based on our systematic review of the literature, we found studies that examined the analytic performance and clinical use of HLA devices and their associated risks. Our literature review showed that FDA-cleared HLA devices appear to be safe and effective, and play an important role to aid donor and recipient matching and disease diagnosis. Device limitations can lead to incorrect HLA testing results that may negatively affect patient care.

Figure 1: Workflow of article selection described in Section 1.1

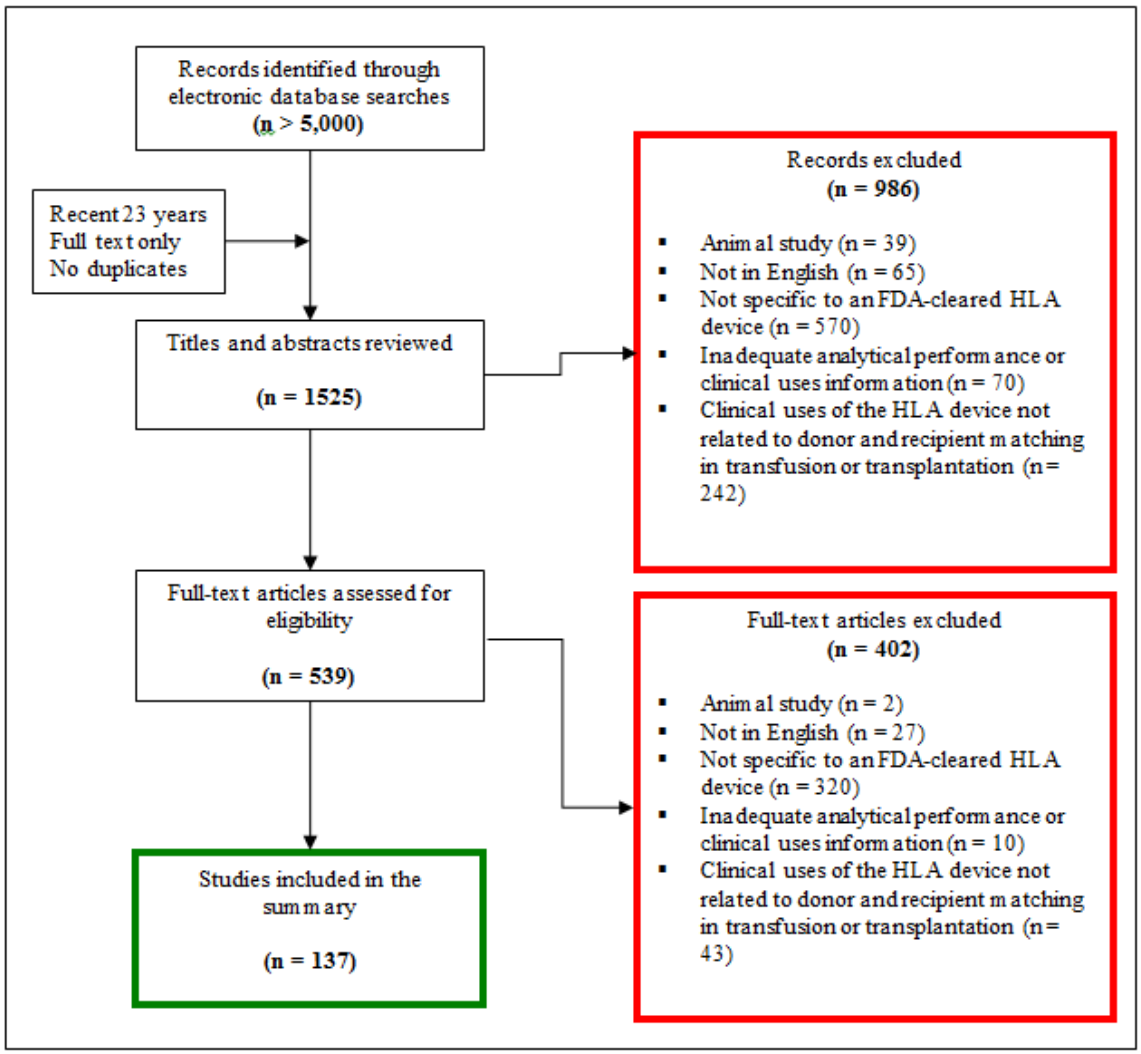
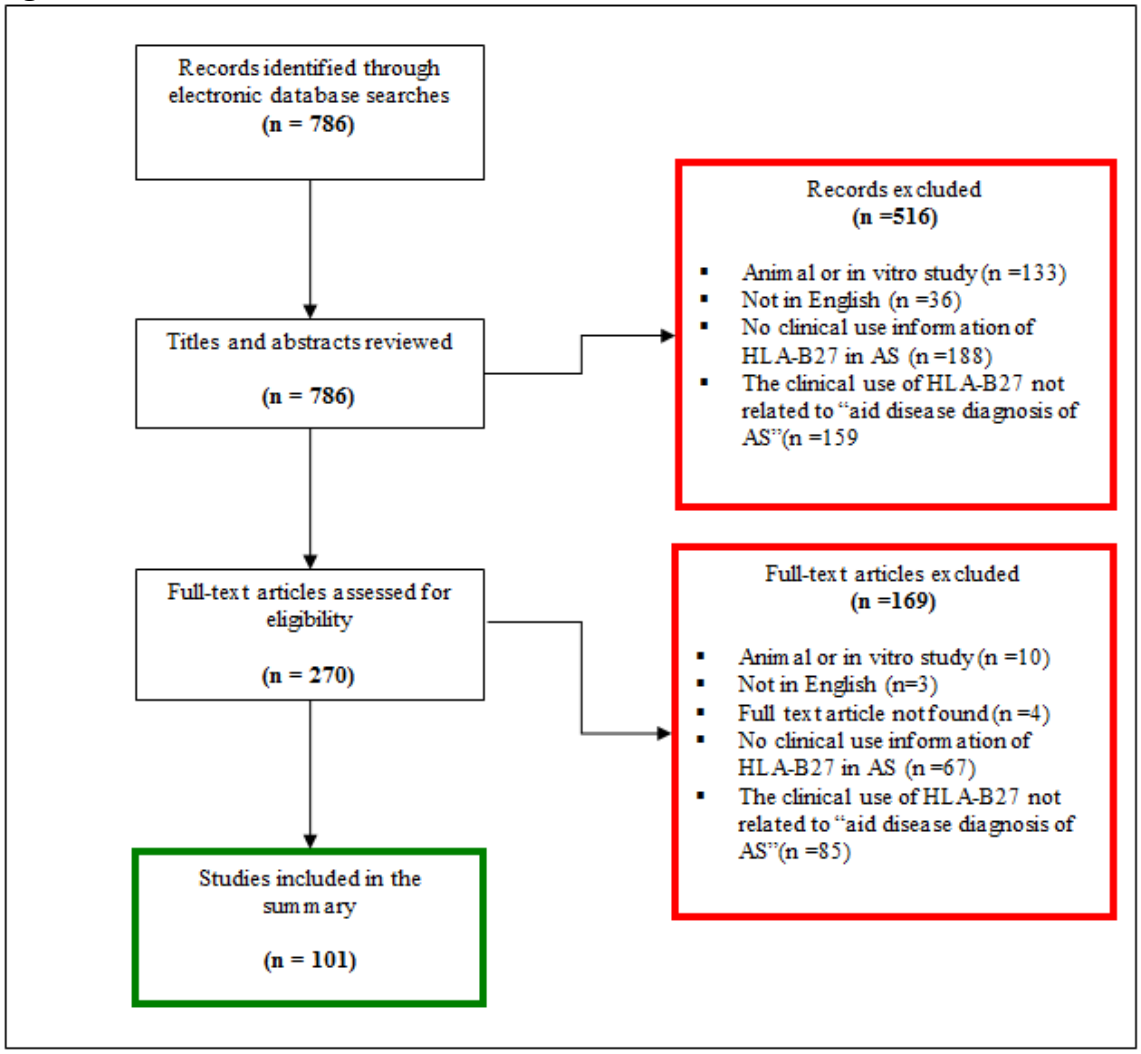


Figure 2: Workflow of article selection described in Section 1.2.



Literature Review Reference List

1. Ayed K, Jendoubi SA, Makhlouf M, Sfar I, Abdallah TB: **Comparison of HLA Class I and II Molecular and Serological Typing within Clinical Laboratory**. *Saudi J Kidney Dis Transpl* 2003, **14**:39-42.
2. Woszczek G, Borowiec M, Mis M, Gorska M, Kowalski ML: **Comparison of serological and molecular (PCR-SSP) techniques of HLA-DR typing in clinical laboratory routine**. *Ann Transplant* 1997, **2**:39-42.
3. Lorentzen DF, Iwanaga KK, Meuer KJ, Moritz TL, Watkins DI: **A 25% error rate in serologic typing of HLA-B homozygotes**. *Tissue Antigens* 1997, **50**:359-365.
4. Bozon MV, Delgado JC, Selvakumar A, Clavijo OP, Salazar M, Ohashi M, Alosco SM, Russell J, Yu N, Dupont B, et al.: **Error rate for HLA-B antigen assignment by serology: implications for proficiency testing and utilization of DNA-based typing methods**. *Tissue Antigens* 1997, **50**:387-394.
5. Noreen HJ, Yu N, Setterholm M, Ohashi M, Baisch J, Endres R, Fernandez-Vina M, Heine U, Hsu S, Kamoun M, et al.: **Validation of DNA-based HLA-A and HLA-B testing of volunteers for a bone marrow registry through parallel testing with serology**. *Tissue Antigens* 2001, **57**:221-229.
6. Gourley IS, Kearns J, McKeen M, Birkos S, Brown M, Kamoun M: **HLA Class I typing of volunteers for a bone marrow registry: QC analysis by DNA-based methodology identifies serological typing discrepancies in the assignment of HLA-A and B antigens**. *Tissue Antigens* 2002, **59**:211-215.
7. Schaffer M, Olerup O: **HLA-AB typing by polymerase-chain reaction with sequence-specific primers: more accurate, less errors, and increased resolution compared to serological typing**. *Tissue Antigens* 2001, **58**:299-307.
8. Jawdat D, Al SS, Sutton P, Al AH, Shubaili A, Uyar FA, Hajeer AH: **HLA-C polymorphisms in two cohorts of donors for bone marrow transplantation**. *Saudi J Kidney Dis Transpl* 2012, **23**:467-470.
9. Adriaanse MP, Vreugdenhil AC, Vastmans V, Groeneveld L, Molenbroeck S, Schott DA, Voorter CE, Tilanus MG: **Human leukocyte antigen typing using buccal swabs as accurate and non-invasive substitute for venipuncture in children at risk for celiac disease**. *J Gastroenterol Hepatol* 2016, **31**:1711-1716.
10. Rahal M, Kervaire B, Villard J, Tiercy JM: **DNA typing by microbead arrays and PCR-SSP: apparent false-negative or -positive hybridization or amplification signals disclose new HLA-B and -DRB1 alleles**. *Tissue Antigens* 2008, **71**:238-241.
11. Gabriel C, Stabentheiner S, Danzer M, Proll J: **What Next? The Next Transit from Biology to Diagnostics: Next Generation Sequencing for Immunogenetics**. *Transfus. Med Hemother* 2011, **38**:308-317.
12. Testi M, Iannelli S, Testa G, Troiano M, Capelli S, Fruet F, Federici G, Bontadini A, Andreani M: **Evaluation of DRB1 high resolution typing by a new SSO-based Luminex method**. *Mol Biol Rep* 2012, **39**:13-16.
13. Corell A, Pay AL, Little AM, Hoddinott MA, Arguello JR, Borton M, Dunne C, Ogilvie H, O'Shea J, Madrigal JA, et al.: **Reference strand mediated conformation analysis resolves HLA-DRB1 typing ambiguities when matching for unrelated bone marrow transplantation**. *Tissue Antigens* 2000, **56**:82-86.
14. Roh EY, In JW, Shin S, Yoon JH, Park KU, Song EY: **Performance of LIFECODES HLA-DQB1 typing kit using Luminex platform in Koreans**. *Ann Lab. Med* 2015, **35**:123-127.
15. Linjama T, Impola U, Niittyvuopio R, Kuittinen O, Kaare A, Rimpilainen J, Volin L, Perasaari J, Jaatinen T, Lauronen J, et al.: **Conflicting HLA assignment by three different typing methods due to the apparent loss of heterozygosity in the MHC region**. *HLA* 2016, **87**:350-355.
16. Tait BD, Hudson F, Cantwell L, Brewin G, Holdsworth R, Bennett G, Jose M: **Review article: Luminex technology for HLA antibody detection in organ transplantation**. *Nephrology (Carlton.)* 2009, **14**:247-254.

17. Heinemann FM: **HLA Genotyping and Antibody Characterization Using the Luminex Multiplex Technology**. *Transfus. Med Hemother* 2009, **36**:273-278.
18. Minucci PB, Grimaldi V, Casamassimi A, Cacciatore F, Sommese L, Picascia A, Resse M, Sabia C, Russo A, Napoli C: **Methodologies for anti-HLA antibody screening in patients awaiting kidney transplant: a comparative study**. *Exp. Clin Transplant* 2011, **9**:381-386.
19. Moszkowska G, Zielinska H, Zielinski M, Dukat-Mazurek A, Debska-Slizien A, Rutkowski B, Lewandowska D, Danielewicz R, Trzonkowski P: **Identification of patients with increased immunological risk among potential kidney recipients in the Polish population**. *Human Immunology* 2014, **75**:650-655.
20. Castillo-Rama M, Castro MJ, Bernardo I, Meneu-Diaz JC, Elola-Olaso AM, Calleja-Antolin SM, Romo E, Morales P, Moreno E, Paz-Artal E: **Preformed antibodies detected by cytotoxic assay or multibead array decrease liver allograft survival: role of human leukocyte antigen compatibility**. *Liver Transpl* 2008, **14**:554-562.
21. Gombos P, Opelz G, Scherer S, Morath C, Zeier M, Schemmer P, Susal C: **Influence of test technique on sensitization status of patients on the kidney transplant waiting list**. *Am J Transplant* 2013, **13**:2075-2082.
22. Monteiro F, Rodrigues H, Kalil J, Castro MC, Panajotopoulos N, Paredes M, Massarolo P, Mies S: **Pre- and posttransplant monitoring of alloantibodies by complement-dependent cytotoxicity and luminex methodologies in liver transplantation**. *Transplant. Proc* 2012, **44**:2411-2412.
23. Schlaf G, Pollok-Kopp B, Manzke T, Schurat O, Altermann W: **Novel solid phase-based ELISA assays contribute to an improved detection of anti-HLA antibodies and to an increased reliability of pre-and post-transplant crossmatching**. *NDT Plus* 2010, **3**:527-538.
24. Colombo MB, Haworth SE, Poli F, Nocco A, Puglisi G, Innocente A, Serafini M, Messa P, Scalamogna M: **Luminex technology for anti-HLA antibody screening: evaluation of performance and of impact on laboratory routine**. *Cytometry. B Clin Cytom* 2007, **72**:465-471.
25. Kurtulmus Y, Ayna TK, Soyoz M, Ozyilmaz B, Tanrisev M, Afacan G, Colak H, Pirim I: **Comparision of anti-HLA antibodies of kidney transplant candidates with chronic renal failure by two different methods: Flow-PRA and Luminex PRA**. *Transplant. Proc* 2013, **45**:875-877.
26. Fadeyi E, Adams S, Peterson B, Hackett J, Byrne P, Klein HG, Marincola FM, Leitman SF, Stroncek DF: **Analysis of a high-throughput HLA antibody screening assay for use with platelet donors**. *Transfusion* 2008, **48**:1174-1179.
27. Lee PC, Ozawa M, Hung CJ, Lin YJ, Chang SS, Chou TC: **Reappraisal of HLA antibody analysis and crossmatching in kidney transplantation**. *Transplant. Proc* 2009, **41**:95-98.
28. Eckels DD, Stehlik J, Kfoury AG: **The detection and role of circulating antibodies in rejection**. *Current Opinion in Organ Transplantation* 2013, **18**:589-594.
29. Batal I, Zeevi A, Lunz JG, III, Aggarwal N, Shapiro R, Randhawa P, Girnita A: **Antihuman leukocyte antigen-specific antibody strength determined by complement-dependent or solid-phase assays can predict positive donor-specific crossmatches**. *Arch Pathol Lab Med* 2010, **134**:1534-1540.
30. Zachary AA, Sholander JT, Houp JA, Leffell MS: **Using real data for a virtual crossmatch**. *Hum Immunol* 2009, **70**:574-579.
31. Visentin J, Guidicelli G, Moreau JF, Lee JH, Taupin JL: **Deciphering allogeneic antibody response against native and denatured HLA epitopes in organ transplantation**. *Eur J Immunol* 2015, **45**:2111-2121.
32. Schinstock CA, Gandhi MJ, Stegall MD: **Interpreting Anti-HLA Antibody Testing Data: A Practical Guide for Physicians**. *Transplantation* 2016, **100**:1619-1628.
33. Oaks M, Michel K, Sulemanjee NZ, Thohan V, Downey FX: **Practical value of identifying antibodies to cryptic HLA epitopes in cardiac transplantation**. *J Heart Lung Transplant* 2014, **33**:713-720.
34. Visentin J, Guidicelli G, Bachelet T, Jacquelinet C, Audry B, Nong T, Dubois V, Moreau JF, Lee JH, Couzi L, et al.: **Denatured class I human leukocyte antigen antibodies in sensitized kidney recipients: prevalence, relevance, and impact on organ allocation**. *Transplantation* 2014, **98**:738-744.
35. Visentin J, Guidicelli G, Nong T, Moreau JF, Merville P, Couzi L, Lee JH, Taupin JL: **Evaluation of the iBeads assay as a tool for identifying class I HLA antibodies**. *Hum Immunol* 2015, **76**:651-656.

36. Jacob EK, De Goey SR, Gandhi MJ: **Positive virtual crossmatch with negative flow crossmatch results in two cases**. *Transpl. Immunol* 2011, **25**:77-81.
37. Visentin J, Marroc M, Guidicelli G, Bachelet T, Nong T, Moreau JF, Lee JH, Merville P, Couzi L, Taupin JL: **Clinical impact of preformed donor-specific denatured class I HLA antibodies after kidney transplantation**. *Clin Transplant* 2015, **29**:393-402.
38. Grenzi PC, de MR, Silva RZ, Campos EF, Gerbase-DeLima M: **Antibodies against denatured HLA class II molecules detected in luminex-single antigen assay**. *Hum Immunol* 2013, **74**:1300-1303.
39. Gao B, Rong C, Porcheray F, Moore C, Girouard TC, Saidman SL, Wong W, Fu Y, Zorn E: **Evidence to Support a Contribution of Polyreactive Antibodies to HLA Serum Reactivity**. *Transplantation* 2016, **100**:217-226.
40. Kosmoliaptsis V, Bradley JA, Peacock S, Chaudhry AN, Taylor CJ: **Detection of immunoglobulin G human leukocyte antigen-specific alloantibodies in renal transplant patients using single-antigen-beads is compromised by the presence of immunoglobulin M human leukocyte antigen-specific alloantibodies**. *Transplantation* 2009, **87**:813-820.
41. Kosmoliaptsis V, O'Rourke C, Bradley JA, Taylor CJ: **Improved Luminex-based human leukocyte antigen-specific antibody screening using dithiothreitol-treated sera**. *Hum Immunol* 2010, **71**:45-49.
42. Katalinic N, Fucak M, Crnic T, Curkovic M, Starcevic A, Balen S: **Pretransplantation monitoring of HLA antibodies by complement dependent cytotoxicity and Luminex-based assays**. *Wien Klin Wochenschr* 2017, **129**:33-37.
43. Visentin J, Guidicelli G, Couzi L, Merville P, Lee JH, Di Primo C, Taupin JL: **Deciphering IgM interference in IgG anti-HLA antibody detection with flow beads assays**. *Hum Immunol* 2016, **77**:1048-1054.
44. Schnaidt M, Weinstock C, Jurisic M, Schmid-Horch B, Ender A, Wernet D: **HLA antibody specification using single-antigen beads--a technical solution for the prozone effect**. *Transplantation* 2011, **92**:510-515.
45. Anani WQ, Zeevi A, Lunz JG: **EDTA Treatment of Serum Unmasks Complement-Mediated Prozone Inhibition in Human Leukocyte Antigen Antibody Testing**. *Am J Clin Pathol* 2016, **146**:346-352.
46. Zachary AA, Lucas DP, Detrick B, Leffell MS: **Naturally occurring interference in Luminex assays for HLA-specific antibodies: characteristics and resolution**. *Hum Immunol* 2009, **70**:496-501.
47. Gloor JM, Moore SB, Schneider BA, DeGoey SR, Stegall MD: **The effect of antithymocyte globulin on anti-human leukocyte antigen antibody detection assays**. *Transplantation* 2007, **84**:258-264.
48. Lee N, Park HS, In JW, Roh EY, Shin S, Park KU, Song EY: **Association of HLA types with non-specific binding of negative control beads in luminex panel reactive antibody (PRA) screening assay**. *Clinical Laboratory* 2017, **63**:169-174.
49. Reed EF, Rao P, Zhang Z, Gebel H, Bray RA, Guleria I, Lunz J, Mohanakumar T, Nickerson P, Tambur AR, et al.: **Comprehensive assessment and standardization of solid phase multiplex-bead arrays for the detection of antibodies to HLA**. *Am J Transplant* 2013, **13**:1859-1870.
50. Gandhi MJ, Carrick DM, Jenkins S, De GS, Ploeger NA, Wilson GA, Lee JH, Winters JL, Stubbs JR, Toy P, et al.: **Lot-to-lot variability in HLA antibody screening using a multiplexed bead-based assay**. *Transfusion* 2013, **53**:1940-1947.
51. Friedlander R, Putheti P, Diaz E, Menon A, Ponce B, Muthukumar T, Sharma VK, Dadhania D, Suthanthiran M: **On the detection of anti-HLA antibodies using single antigen bead Luminex assay: lot-to-lot variations in MFI**. *Transplantation* 2013, **96**:e24-e26.
52. Gandhi MJ, Degoey S, Falbo D, Jenkins S, Stubbs JR, Noreen H, Lorentzen DF, Lee J, Stegall M: **Inter and intra laboratory concordance of HLA antibody results obtained by single antigen bead based assay**. *Hum Immunol* 2013, **74**:310-317.
53. Lachmann N, Todorova K, Schulze H, Schonemann C: **Luminex((R)) and its applications for solid organ transplantation, hematopoietic stem cell transplantation, and transfusion**. *Transfus. Med Hemother* 2013, **40**:182-189.
54. Kamburova EG, Wisse BW, Joosten I, Allebes WA, van der Meer A, Hilbrands LB, Baas MC, Spierings E, Hack CE, van Reekum FE, et al.: **How can we reduce costs of solid-phase multiplex-bead assays used to determine anti-HLA antibodies?** *HLA* 2016, **88**:110-119.

55. Kao GS, Wood IG, Dorfman DM, Milford EL, Benjamin RJ: **Investigations into the role of anti-HLA class II antibodies in TRALI**. *Transfusion* 2003, **43**:185-191.
56. Uboldi de CM, Pratico L, Curtoni ES: **Comparison of different techniques for detection of anti-HLA antibodies in sera from patients awaiting kidney transplantation**. *Eur J Immunogenet* 2002, **29**:379-382.
57. Moore SB, Ploeger NA, DeGoey SR: **HLA antibody screening: comparison of a solid phase enzyme-linked immunoassay with antiglobulin-augmented lymphocytotoxicity**. *Transplantation* 1997, **64**:1617-1620.
58. Worthington JE, Thomas AA, Dyer PA, Martin S: **Detection of HLA-specific antibodies by PRA-STAT and their association with transplant outcome**. *Transplantation* 1998, **65**:121-125.
59. Monteiro F, Buelow R, Mineiro C, Rodrigues H, Kalil J: **Identification of patients at high risk of graft loss by pre- and posttransplant monitoring of anti-HLA class I IgG antibodies by enzyme-linked immunosorbent assay**. *Transplantation* 1997, **63**:542-546.
60. Vojvodic S, Ademovic-Sazdanic D: **HLA antibody screening strategy in patients awaiting kidney transplantation**. *Central European Journal of Medicine* 2013, **8**:317-321.
61. Gebel HM, Bray RA: **Sensitization and sensitivity: defining the unsensitized patient**. *Transplantation* 2000, **69**:1370-1374.
62. Worthington JE, Robson AJ, Sheldon S, Langton A, Martin S: **A comparison of enzyme-linked immunoabsorbent assays and flow cytometry techniques for the detection of HLA specific antibodies**. *Hum Immunol* 2001, **62**:1178-1184.
63. Pierquin A, Masson D, Toupance O, Tabary T, Bougy F, Bensa JC, Chanard J, Cohen JH: **False-negative results in anti-HLA antibody detection by Sangstat ELISA: complement dependent cytotoxicity is not yet obsolete**. *Transplantation* 1996, **62**:1533-1534.
64. Harmer AW, Heads AJ, Vaughn RW: **Detection of HLA class I- and class II-specific antibodies by flow cytometry and PRA-STAT screening in renal transplant recipients**. *Transplantation* 1997, **63**:1828-1832.
65. Dunbar NM, Yazer MH, Bravo MD, Kamel HT, Gorlin J, Norris PJ, Williamson PC, Vassallo RR: **An association between ABO group and HLA antibody detection**. *Transfusion* 2017, **57**:313-318.
66. Schlaf G, Pollok-Kopp B, Altermann WW: **Sensitive solid-phase detection of donor-specific antibodies as an aid highly relevant to improving allograft outcomes**. *Molecular Diagnosis and Therapy* 2014, **18**:185-201.
67. Altermann WW, Seliger B, Sel S, Wendt D, Schlaf G: **Comparison of the established standard complement-dependent cytotoxicity and flow cytometric crossmatch assays with a novel ELISA-based HLA crossmatch procedure**. *Histol. Histopathol* 2006, **21**:1115-1124.
68. Yang CW, Oh EJ, Lee SB, Moon IS, Kim DG, Choi BS, Park SC, Choi YJ, Park YJ, Han K: **Detection of donor-specific anti-HLA class I and II antibodies using antibody monitoring system**. *Transplant. Proc* 2006, **38**:2803-2806.
69. Hwang HS, Hyoung BJ, Lee SY, Jeon YJ, Yoon HE, Kim JY, Choi BS, Oh EJ, Kim YS, Bang BK, et al.: **Comparison of antibody monitoring system with flow cytometric crossmatch test in renal transplant recipients with high panel-reactive antibody**. *Nephron. Clin Pract* 2009, **111**:c260-c264.
70. Buelow R, Chiang TR, Monteiro F, Cornejo MC, Ellingson L, Claas F, Gaber O, Gelder F, Kotb M, Orosz C, et al.: **Soluble HLA antigens and ELISA--a new technology for crossmatch testing**. *Transplantation* 1995, **60**:1594-1599.
71. Aung FM, Cano P, Fernandez-Vina M, Lichtiger B: **Results of HLA antibody testing using ELISA vs the fluorescent bead method and retrospective review of data for recipients of packed RBCs and platelets from male HLA-immunized donors**. *Am J Clin Pathol* 2011, **135**:90-95.
72. Pourfarziani V, Einollahi B, Assari S, Kardavani B, Lankarani MM, Kalantar E: **A link between the outcome of living unrelated kidney transplantation and HLA compatibility: A preliminary report**. *Archives of Medical Science* 2007, **3**:108-111.
73. Rodriguez DS, Jankowska-Gan E, Haynes LD, Leverson G, Munoz A, Heisey D, Sollinger HW, Burlingham WJ: **Immune regulation and graft survival in kidney transplant recipients are both enhanced by human leukocyte antigen matching**. *Am J Transplant* 2004, **4**:537-543.
74. Tajik N, Singal D, Pourmand G, Ebrahimi-Rad M, Radjabzadeh M, Tavasoli P, Khosravi F, Nikbin B: **Prospective study of microchimerism in renal allograft recipients: association between HLA-DR matching, microchimerism and acute rejection**. *Clin Transplant* 2001, **15**:192-198.

75. Caro-Oleas JL, Gonzalez-Escribano MF, Gonzalez-Roncero FM, Acevedo-Calado MJ, Cabello-Chaves V, Gentil-Govantes MA, Nunez-Roldan A: **Clinical relevance of HLA donor-specific antibodies detected by single antigen assay in kidney transplantation**. *Nephrol. Dial. Transplant* 2012, **27**:1231-1238.
76. Gilbert M, Paul S, Perrat G, Giannoli C, Pouteil NC, Morelon E, Rigal D, Dubois V: **Impact of pretransplant human leukocyte antigen-C and -DP antibodies on kidney graft outcome**. *Transplant Proc* 2011, **43**:3412-3414.
77. Muro M, Lopez-Alvarez MR, Campillo JA, Marin L, Moya-Quiles MR, Bolarin JM, Botella C, Salgado G, Martinez P, Sanchez-Bueno F, et al.: **Influence of human leukocyte antigen mismatching on rejection development and allograft survival in liver transplantation: is the relevance of HLA-A locus matching being underestimated?** *Transpl Immunol* 2012, **26**:88-93.
78. Na GH, Kim EY, Hong TH, You YK, Kim DG: **Effects of Preoperative Positive Cross-Match and HLA Mismatching on Early Acute Cellular Rejection and Graft Survival in Living Donor Liver Transplantation**. *Ann Transplant* 2015, **20**:553-560.
79. Schulman LL, Weinberg AD, McGregor CC, Suciu-Foca NM, Itescu S: **Influence of donor and recipient HLA locus mismatching on development of obliterative bronchiolitis after lung transplantation**. *Am J Respir. Crit. Care Med* 2001, **163**:437-442.
80. Giebel S, Giorgiani G, Martinetti M, Zecca M, Maccario R, Salvaneschi L, Holowiecki J, Locatelli F: **Low incidence of severe acute graft-versus-host disease in children given haematopoietic stem cell transplantation from unrelated donors prospectively matched for HLA class I and II alleles with high-resolution molecular typing**. *Bone Marrow Transplant* 2003, **31**:987-993.
81. El KN, Legouvello S, Joseph CM, Salesses P, Rieux C, Cordonnier C, Vernant JP, Farcet JP, Bierling P, Kuentz M: **High resolution HLA class I and II typing and CTLp frequency in unrelated donor transplantation: a single-institution retrospective study of 69 BMTs**. *Bone Marrow Transplant* 2001, **27**:35-43.
82. Huo MR, Xu LP, Li D, Liu DH, Liu KY, Chen H, Han W, Chen YH, Wang Y, Wang JZ, et al.: **The effect of HLA disparity on clinical outcome after HLA-haploidentical blood and marrow transplantation**. *Clin. Transplant* 2012, **26**:284-291.
83. Loiseau P, Esperou H, Busson M, Sghiri R, Tamouza R, Hilarius M, Raffoux C, Devergie A, Ribaud P, Socie G, et al.: **DPB1 disparities contribute to severe GVHD and reduced patient survival after unrelated donor bone marrow transplantation**. *Bone Marrow Transplant* 2002, **30**:497-502.
84. Burt C, Parker A, McQuaker G, Copland M, Brierley C, Little AM, Clark A: **In a 12-allele analysis HLA-DPB1 matching is associated with improved OS in leukaemic and myelodysplastic patients receiving myeloablative T-cell-depleted PBSCT from unrelated donors**. *Bone. Marrow. Transplant* 2014, **49**:657-663.
85. Pan Z, Yuan X, Li Y, Wu X, Zhu W, Bao X, Zhao Q, He J: **Dynamic Detection of Anti-Human Leukocyte Antigen (HLA) Antibodies but not HLA-DP Loci Mismatches Can Predict Acute Graft-versus-Host Disease and Overall Survival in HLA 12/12-Matched Unrelated Donor Allogeneic Hematopoietic Stem Cell Transplantation for Hematological Malignancies**. *Biol Blood Marrow Transplant* 2016, **22**:86-95.
86. Sun Y, Zhao D, Jin L, Liu N, Liang F, Kong F, Xi Y: **Human leukocyte antigens A and B Loci genotyping by reference strand-mediated conformation analysis in hematopoietic stem cell transplantation donor selection**. *Int J Hematol* 2007, **86**:77-83.
87. Elia L, Arcese W, Torello M, Iori AP, Guglielmi C, Perrone MP, Screnci M, Sprovieri T, Rapanotti MC, Cimino G: **HLA-C and HLA-DQB1 compatibility in unrelated cord blood transplants**. *Haematologica* 1999, **84**:530-534.
88. Xia WJ, Ye X, Tian LW, Xu XZ, Chen YK, Luo GP, Bei CH, Deng J, Santoso S, Fu YS: **Establishment of platelet donor registry improves the treatment of platelet transfusion refractoriness in Guangzhou region of China**. *Transfus. Med* 2010, **20**:269-274.
89. Gingu C, Moise A, Constantinescu I, Serbanescu B, Surcel C, Sinescu I: **Cytotoxic antibodies monitoring in kidney transplantation--their clinical relevance and challenges**. *Rom. J Morphol. Embryol* 2012, **53**:515-519.
90. Haarberg KMK, Tambur AR: **Detection of donor-specific antibodies in kidney transplantation**. *British Medical Bulletin* 2014, **110**:23-34.

91. Gibney EM, Cagle LR, Freed B, Warnell SE, Chan L, Wiseman AC: **Detection of donor-specific antibodies using HLA-coated microspheres: another tool for kidney transplant risk stratification**. *Nephrol. Dial. Transplant* 2006, **21**:2625-2629.
92. Tian J, Li D, Alberghini TV, Rewinski M, Guo N, Bow LM: **Pre-transplant low level HLA antibody shows a composite poor outcome in long-term outcome of renal transplant recipients**. *Ren. Fail* 2015, **37**:198-202.
93. Higgins R, Lowe D, Hathaway M, Williams C, Lam FT, Kashi H, Tan LC, Imray C, Fletcher S, Chen K, et al.: **Human leukocyte antigen antibody-incompatible renal transplantation: excellent medium-term outcomes with negative cytotoxic crossmatch**. *Transplantation* 2011, **92**:900-906.
94. Khovanova N, Daga S, Shaikhina T, Krishnan N, Jones J, Zehnder D, Mitchell D, Higgins R, Briggs D, Lowe D: **Subclass analysis of donor HLA-specific IgG in antibody-incompatible renal transplantation reveals a significant association of IgG4 with rejection and graft failure**. *Transpl Int* 2015, **28**:1405-1415.
95. Chung BH, Kim JH, Choi BS, Park CW, Kim JI, Moon IS, Kim YS, Choi YJ, Oh EJ, Yang CW: **Clinical significance of the presence of anti-human leukocyte antigen-donor specific antibody in kidney transplant recipients with allograft dysfunction**. *Korean J Intern Med* 2016.
96. Ling M, Marfo K, Masiakos P, Aljanabi A, Lindower J, Glicklich D, de BG, Greenstein S, Chapochnick-Friedmann J, Kayler L, et al.: **Pretransplant anti-HLA-Cw and anti-HLA-DP antibodies in sensitized patients**. *Hum Immunol* 2012, **73**:879-883.
97. Bostock IC, Alberu J, Arvizu A, Hernandez-Mendez EA, De-Santiago A, Gonzalez-Tableros N, Lopez M, Castelan N, Contreras AG, Morales-Buenrostro LE, et al.: **Probability of deceased donor kidney transplantation based on % PRA**. *Transplant Immunology* 2013, **28**:154-158.
98. Picascia A, Grimaldi V, Sabia C, Napoli C: **Comprehensive assessment of sensitizing events and anti-HLA antibody development in women awaiting kidney transplantation**. *Transpl Immunol* 2016, **36**:14-19.
99. Akgul SU, Ciftci HS, Temurhan S, Caliskan Y, Bayraktar A, Tefik T, Kaya IA, Canitez IO, Demir E, Yazici H, et al.: **Association Between HLA Antibodies and Different Sensitization Events in Renal Transplant Candidates**. *Transplantation Proceedings* 2017, **49**:425-429.
100. Ishida H, Tanabe K, Furusawa M, Ishizuka T, Hayashi T, Tokumoto T, Miyamoto N, Shirakawa H, Shimmura H, Shimizu T, et al.: **Evaluation of flow cytometric panel reactive antibody in renal transplant recipients - examination of 238 cases of renal transplantation**. *Transpl. Int* 2005, **18**:163-168.
101. Li L-Y, Liu Z-G, Chen J-R, Qian J, Sun E-W, Zhao M: **Monitoring human leukocyte antigen humoral immunity sensitization in renal transplant recipients and its clinical significance**. *Journal of Clinical Rehabilitative Tissue Engineering Research* 2008, **12**:3561-3566.
102. Meng HL, Jin XB, Li XT, Wang HW, Lu JJ: **Impact of human leukocyte antigen matching and recipients' panel reactive antibodies on two-year outcome in presensitized renal allograft recipients**. *Chin. Med J (Engl.)* 2009, **122**:420-426.
103. Christiaans MH, Nieman F, van Hooff JP, EM vdB-L: **Detection of HLA class I and II antibodies by ELISA and complement-dependent cytotoxicity before and after transplantation**. *Transplantation* 2000, **69**:917-927.
104. Lee PC, Chen YL, Chou TC, Wang WM, Wang JD, Hung CJ, Chang SS, Lin YJ, Chan RH: **The clinical significance of human leukocyte antigen antibody development in kidney transplantation**. *Transplant. Proc* 2012, **44**:264-266.
105. Raess M, Frohlich G, Roos M, Rusi B, Wilhelm MJ, Noll G, Ruschitzka F, Fehr T, Enseleit F: **Donor-specific anti-HLA antibodies detected by Luminex: predictive for short-term but not long-term survival after heart transplantation**. *Transpl. Int* 2013, **26**:1097-1107.
106. Gandhi MJ, DeGoey SR, Bundy K, Kremers WK, Knauer R, Pereira N, Edwards B, Kushwaha S, Daly RC: **Effect of pretransplant human leukocyte antigen antibodies detected by solid-phase assay on heart transplant outcomes**. *Transplant. Proc* 2011, **43**:3840-3846.
107. Svobodova E, Gazdic T, Kubanek M, Vymetalova J, Voska L, Kment M, Lanska V, Kolesar L, Urban M, Netuka I, et al.: **Novel insights into pretransplant allosensitization in heart transplant recipients in the contemporary era of immunosuppression and rejection surveillance**. *Transpl Int* 2016, **29**:63-72.

108. Zangwill S, Ellis T, Stendahl G, Zahn A, Berger S, Tweddell J: **Practical application of the virtual crossmatch**. *Pediatr. Transplant* 2007, **11**:650-654.
109. Wozniak LJ, Naini BV, Hickey MJ, Bhattacharyya S, Reed EF, Busuttil RW, Farmer DG, Vargas JH, Venick RS, McDiarmid SV: **Acute antibody-mediated rejection in ABO-compatible pediatric liver transplant recipients: case series and review of the literature**. *Pediatric Transplantation* 2017, **21**.
110. Bishara A, Brautbar C, Eid A, Scherman L, Ilan Y, Safadi R: **Is presensitization relevant to liver transplantation outcome?** *Hum Immunol* 2002, **63**:742-750.
111. Smith JD, Ibrahim MW, Newell H, Danskine AJ, Soresi S, Burke MM, Rose ML, Carby M: **Pre-transplant donor HLA-specific antibodies: Characteristics causing detrimental effects on survival after lung transplantation**. *Journal of Heart and Lung Transplantation* 2014, **33**:1074-1082.
112. Girnita AL, McCurry KR, Iacono AT, Duquesnoy R, Corcoran TE, Awad M, Spichty KJ, Yousem SA, Burckart G, Dauber JH, et al.: **HLA-specific antibodies are associated with high-grade and persistent-recurrent lung allograft acute rejection**. *J Heart Lung Transplant* 2004, **23**:1135-1141.
113. Brugiere O, Suberbielle C, Thabut G, Lhuillier E, Dauriat G, Metivier AC, Gautreau C, Charron D, Mal H, Parquin F, et al.: **Lung transplantation in patients with pretransplantation donor-specific antibodies detected by Luminex assay**. *Transplantation* 2013, **95**:761-765.
114. Ruiz P, Carreno M, Weppler D, Gomez C, Island E, Selvaggi G, Nishida S, Moon J, Levi D, Tekin A, et al.: **Immediate antibody-mediated (hyperacute) rejection in small-bowel transplantation and relationship to cross-match status and donor-specific C4d-binding antibodies: case report**. *Transplant. Proc* 2010, **42**:95-99.
115. Chang YJ, Zhao XY, Xu LP, Zhang XH, Wang Y, Han W, Chen H, Wang FR, Mo XD, Zhang YY, et al.: **Donor-specific anti-human leukocyte antigen antibodies were associated with primary graft failure after unmanipulated haploidentical blood and marrow transplantation: a prospective study with randomly assigned training and validation sets**. *J Hematol Oncol* 2015, **8**:84.
116. Ciurea SO, Thall PF, Wang X, Wang SA, Hu Y, Cano P, Aung F, Rondon G, Molldrem JJ, Korbling M, et al.: **Donor-specific anti-HLA Abs and graft failure in matched unrelated donor hematopoietic stem cell transplantation**. *Blood* 2011, **118**:5957-5964.
117. Takanashi M, Atsuta Y, Fujiwara K, Kodo H, Kai S, Sato H, Kohsaki M, Azuma H, Tanaka H, Ogawa A, et al.: **The impact of anti-HLA antibodies on unrelated cord blood transplantations**. *Blood* 2010, **116**:2839-2846.
118. Delbos F, Barhoumi W, Cabanne L, Beckerich F, Robin C, Redjoul R, Astati S, Toma A, Pautas C, Ansart-Pirenne H, et al.: **Donor Immunization Against Human Leukocyte Class II Antigens is a Risk Factor for Graft-versus-Host Disease**. *Biol Blood Marrow Transplant* 2016, **22**:292-299.
119. Eby BC, Redfield RR, Ellis TM, Leverson GE, Schenian AR, Odorico JS: **Virtual HLA Crossmatching as a Means to Safely Expedite Transplantation of Imported Pancreata**. *Transplantation* 2016, **100**:1103-1110.
120. Lin Y, Saw CL, Hannach B, Goldman M: **Transfusion-related acute lung injury prevention measures and their impact at Canadian Blood Services**. *Transfusion* 2012, **52**:567-574.
121. Carrick DM, Norris PJ, Endres RO, Pandey S, Kleinman SH, Wright D, Sun Y, Busch MP: **Establishing assay cutoffs for HLA antibody screening of apheresis donors**. *Transfusion* 2011, **51**:2092-2101.
122. Imoto S, Kawamura K, Tokumine Y, Araki N, Akita S, Nishimura C, Inaba H, Saigo K, Mabuchi O, Okazaki H: **Acute non-hemolytic transfusion reactions and HLA class I antibody: advantages of solid phase assay compared with conventional complement-dependent assay**. *Transfus. Med* 2010, **20**:95-103.
123. Enein AA, El Desoukey NA, Hussein EA, Hamdi M, Jamjom NA: **HLA alloimmunization in Egyptian aplastic anemia patients receiving exclusively leukoreduced blood components**. *Transfus. Apher. Sci* 2013, **48**:213-218.
124. Balasubramaniam GS, Morris M, Gupta A, Mesa IR, Thuraisingham R, Ashman N: **Allosensitization rate of male patients awaiting first kidney grafts after leuko-depleted blood transfusion**. *Transplantation* 2012, **93**:418-422.

125. Petz LD, Garratty G, Calhoun L, Clark BD, Terasaki PI, Gresens C, Gornbein JA, Landaw EM, Smith R, Cecka JM: **Selecting donors of platelets for refractory patients on the basis of HLA antibody specificity**. *Transfusion* 2000, **40**:1446-1456.
126. Rosenbaum ER, Pandey S, Harville TO, Drobena GA, Cottler-Fox M: **Flow Cytometric Panel-Reactive Antibody Results and the Ability to Find Transfusion-Compatible Platelets after Antibody-Desensitization for Allogeneic Bone Marrow Transplant**. *Ann Clin Lab Sci* 2016, **46**:662-665.
127. Couzi L, Araujo C, Guidicelli G, Bachelet T, Moreau K, Morel D, Robert G, Wallerand H, Moreau JF, Taupin JL, et al.: **Interpretation of positive flow cytometric crossmatch in the era of the single-antigen bead assay**. *Transplantation* 2011, **91**:527-535.
128. Susal C, Ovens J, Mahmoud K, Dohler B, Scherer S, Ruhenstroth A, Tran TH, Heinold A, Opelz G: **No association of kidney graft loss with human leukocyte antigen antibodies detected exclusively by sensitive Luminex single-antigen testing: a Collaborative Transplant Study report**. *Transplantation* 2011, **91**:883-887.
129. Thiyagarajan UM, Bagul A, Frost J, Horsburgh T, Nicholson ML: **Role of human leukocyte antigen, donor-specific antibodies, and their impact in renal transplantation**. *Transplant. Proc* 2012, **44**:1231-1235.
130. Quillen K, Medrano C, Adams S, Peterson B, Hackett J, Leitman SF, Klein HG, Stroncek DF: **Screening plateletpheresis donors for HLA antibodies on two high-throughput platforms and correlation with recipient outcome**. *Transfusion* 2011, **51**:504-510.
131. Otten HG, Verhaar MC, Borst HP, van EM, van Ginkel WG, Hene RJ, van Zuilen AD: **The significance of pretransplant donor-specific antibodies reactive with intact or denatured human leucocyte antigen in kidney transplantation**. *Clin Exp. Immunol* 2013, **173**:536-543.
132. Sicard A, Amrouche L, Suberbielle C, Carmagnat M, Candon S, Thervet E, Delahousse M, Legendre C, Chatenoud L, Snanoudj R: **Outcome of kidney transplantations performed with preformed donor-specific antibodies of unknown etiology**. *Am J Transplant* 2014, **14**:193-201.
133. Morales-Buenrostro LE, Terasaki PI, Marino-Vazquez LA, Lee JH, El-Awar N, Alberu J: **"Natural" human leukocyte antigen antibodies found in nonalloimmunized healthy males**. *Transplantation* 2008, **86**:1111-1115.
134. Vassallo RR, Hsu S, Einarson M, Barone J, Brodsky J, Moroff G: **A comparison of two robotic platforms to screen plateletpheresis donors for HLA antibodies as part of a transfusion-related acute lung injury mitigation strategy**. *Transfusion* 2010, **50**:1766-1777.
135. Roberts T, Tumer G, Gebel HM, Bray RA: **Solid-phase assays for the detection of alloantibody against human leukocyte antigens: Panacea or Pandora?** *International Journal of Immunogenetics* 2014, **41**:362-369.
136. Bray RA, Gebel HM: **Strategies for human leukocyte antigen antibody detection**. *Curr Opin Organ Transplant* 2009, **14**:392-397.
137. Middleton D, Jones J, Lowe D: **Nothing's perfect: The art of defining HLA-specific antibodies**. *Transplant Immunology* 2014, **30**:115-121.
138. Raychaudhuri SP, Deodhar A: **The classification and diagnostic criteria of ankylosing spondylitis**. *J Autoimmun* 2014, **48-49**:128-133.
139. Robinson PC, Brown MA: **The genetics of ankylosing spondylitis and axial spondyloarthritis**. *Rheum Dis Clin North Am* 2012, **38**:539-553.
140. Robinson PC, Brown MA: **Genetics of ankylosing spondylitis**. *Mol Immunol* 2014, **57**:2-11.
141. Dougados M, Baeten D: **Spondyloarthritis**. *Lancet* 2011, **377**:2127-2137.
142. Cauli A, Dessole G, Vacca A, Porru G, Cappai L, Piga M, Bitti PP, Fiorillo MT, Sorrentino R, Carcassi C, et al.: **Susceptibility to ankylosing spondylitis but not disease outcome is influenced by the level of HLA-B27 expression, which shows moderate variability over time**. *Scand J Rheumatol* 2012, **41**:214-218.
143. Cauli A, Piga M, Dessole G, Porru G, Vacca A, Ibba V, Mathieu A: **Genetics of seronegative spondyloarthritis, susceptibility and aetiopathogenesis: A single centre experience**. *Clinical Drug Investigation* 2013, **33**:S104-S106.
144. Barbarino JM, Kroetz DL, Klein TE, Altman RB: **PharmGKB summary: Very important pharmacogene information for human leukocyte antigen B**. *Pharmacogenetics and Genomics* 2015, **25**:205-221.

145. Danve A, O'Dell J: **The ongoing quest for biomarkers in Ankylosing Spondylitis**. *Int J Rheum Dis* 2015, **18**:826-834.
146. Gouveia EB, Elmann D, Morales MS: **Ankylosing spondylitis and uveitis: overview**. *Rev Bras Reumatol* 2012, **52**:742-756.
147. Barczyńska TA, Węgierska M, Zuchowski P, Dura M, Zalewska J, Waszczak M, Jeka S: **Coexistence of rheumatoid arthritis and ankylosing spondylitis**. *Reumatologia* 2015, **53**:279-285.
148. Rashid T, Ebringer A: **Detection of klebsiella antibodies and HLA-B27 allelotypes could be used in the early diagnosis of ankylosing spondylitis with a potential for the use of "Low starch diet" in the treatment**. *Current Rheumatology Reviews* 2012, **8**:109-119.
149. Poddubnyy D: **Improving diagnosis of ankylosing spondylitis and spondyloarthritis in general**. *International Journal of Clinical Rheumatology* 2011, **6**:655-667.
150. Wallis D, Inman RD: **Recognition of preclinical and early disease in axial spondyloarthritis**. *Rheum Dis Clin North Am* 2014, **40**:685-697.
151. MacHado P, Landewé R, Van Der Heijde D: **New developments in the diagnosis and treatment of axial spondyloarthritis**. *Clinical Investigation* 2013, **3**:153-171.
152. Baraliakos X, Braun J: **Non-radiographic axial spondyloarthritis and ankylosing spondylitis: what are the similarities and differences?** *RMD Open* 2015, **1**:e000053.
153. Wallis D, Haroon N, Ayearst R, Carty A, Inman RD: **Ankylosing spondylitis and nonradiographic axial spondyloarthritis: part of a common spectrum or distinct diseases?** *J Rheumatol* 2013, **40**:2038-2041.
154. Akassou A, Yacoubi H, Jamil A, Dakka N, Amzazi S, Sadki K, Niamane R, Elhassani S, Bakri Y: **Prevalence of HLA-B27 in Moroccan healthy subjects and patients with ankylosing spondylitis and mapping construction of several factors influencing AS diagnosis by using multiple correspondence analysis**. *Rheumatol Int* 2015, **35**:1889-1894.
155. Rashid T, Wilson C, Ebringer A: **Raised incidence of ankylosing spondylitis among Inuit populations could be due to high HLA-B27 association and starch consumption**. *Rheumatol Int* 2015, **35**:945-951.
156. Reveille JD: **Biomarkers for diagnosis, monitoring of progression, and treatment responses in ankylosing spondylitis and axial spondyloarthritis**. *Clin Rheumatol* 2015, **34**:1009-1018.
157. Neerinckx B, Lories RJ: **Emerging concepts in ankylosing spondylitis**. *International Journal of Clinical Rheumatology* 2012, **7**:515-526.
158. Bandinelli F, Salvadorini G, Sedie AD, Riente L, Bombardieri S, Matucci-Cerinic M: **Impact of gender, work, and clinical presentation on diagnostic delay in Italian patients with primary ankylosing spondylitis**. *Clin Rheumatol* 2015.
159. Kassimos DG, Vassilakos J, Magiorkinis G, Garyfallos A: **Prevalence and clinical manifestations of ankylosing spondylitis in young Greek males**. *Clin Rheumatol* 2014, **33**:1303-1306.
160. Popa OM, Bojinca M, Bojinca V, Ciofu C, Dutescu MI, Bardan A, Sfrent-Cornateanu R, Petrek M, Bara C, Popa L: **Distribution of HLA-B27 in Romanian spondyloarthritides patients**. *Int J Immunogenet* 2010, **37**:513-516.
161. Benegas M, Munoz-Gomariz E, Font P, Burgos-Vargas R, Chaves J, Palleiro D, Maldonado Cocco J, Gutierrez M, Saenz R, Steckmen I, et al.: **Comparison of the clinical expression of patients with ankylosing spondylitis from Europe and Latin America**. *J Rheumatol* 2012, **39**:2315-2320.
162. Zholobova YS, Bobyleva VN, Chebysheva SN, Meleshkina AV: **Early diagnosis of juvenile ankylosing spondyloarthritis**. *New Armenian Medical Journal* 2016, **10**:34-43.
163. Liu Y, Li L, Shi S, Chen X, Gao J, Zhu M, Yuan J: **Association study of ankylosing spondylitis and polymorphisms in ERAP1 gene in Zhejiang Han Chinese population**. *Rheumatol Int* 2015.
164. Yi L, Wang J, Guo X, Espitia MG, Chen E, Assassi S, Jin L, Zou H, Reveille JD, Zhou X: **Profiling of hla-B alleles for association studies with ankylosing spondylitis in the chinese population**. *Open Rheumatol J* 2013, **7**:51-54.
165. Ho HH, Chen JY: **Ankylosing spondylitis: Chinese perspective, clinical phenotypes, and associated extra-articular systemic features**. *Curr Rheumatol Rep* 2013, **15**:344.
166. Zou HY, Yu WZ, Wang Z, He J, Jiao M: **Human leukocyte antigen-B27 alleles in Xinjiang Uygur patients with ankylosing spondylitis**. *Genet Mol Res* 2015, **14**:5652-5657.
167. Wei JC, Sung-Ching HW, Hsu YW, Wen YF, Wang WC, Wong RH, Lu HF, Gaalen FA, Chang WC: **Interaction between HLA-B60 and HLA-B27 as a Better Predictor of Ankylosing Spondylitis in a Taiwanese Population**. *PLoS One* 2015, **10**:e0137189.

168. Ma HJ, Yin QF, Hu FP, Guo MH, Liu XD, Liu Y, Xu QY: **Different clinical features in patients with ankylosing spondylitis from southern and northern China**. *Int J Rheum Dis* 2012, **15**:154-162.
169. Otsuka A, Morita M, Yamada H: **Clinical characteristics of Japanese patients with axial spondyloarthritis, and short-term efficacy of adalimumab**. *J Orthop Sci* 2015, **20**:1070-1077.
170. Hajialilo M, Ghorbanihaghjo A, Khabbazi A, Kolahi S, Rashtchizadeh N: **Ankylosing spondylitis in iran; late diagnosis and its causes**. *Iran Red Crescent Med J* 2014, **16**:e11798.
171. Jamshidi AR, Shahlaee A, Farhadi E, Fallahi S, Nicknam MH, Bidad K, Barghamadi M, Mahmoudi M: **Clinical characteristics and medical management of Iranian patients with ankylosing spondylitis**. *Mod Rheumatol* 2014, **24**:499-504.
172. Vojvodic S, Ademovic-Sazdanic D, Busarcevic s: **Human leukocyte antigen-b27 and disease susceptibility in vojvodina, serbia**. *Balkan J Med Genet* 2012, **15**:55-60.
173. Mishra MN, Singal V: **Human leukocyte antigen B27 in 453 Asian Indian patients with seronegative spondyloarthropathy**. *Iran J Immunol* 2010, **7**:252-256.
174. Omair MA, AlDuraibi FK, Bedaiwi MK, Abdulaziz S, Husain W, El Dessougi M, Alhumaidan H, Al Khabbaz HJ, Alahmadi I, Omair MA, et al.: **Prevalence of HLA-B27 in the general population and in patients with axial spondyloarthritis in Saudi Arabia**. *Clin Rheumatol* 2017, **36**:1537-1543.
175. Mustafa KN, Hammoudeh M, Khan MA: **HLA-B27 Prevalence in Arab Populations and Among Patients with Ankylosing Spondylitis**. *J Rheumatol* 2012, **39**:1675-1677.
176. Abdelrahman MH, Mahdy S, Khanjar IA, Siam AM, Malallah HA, Al-Emadi SA, Sarakbi HA, Hammoudeh M: **Prevalence of HLA-B27 in Patients with Ankylosing Spondylitis in Qatar**. *Int J Rheumatol* 2012, **2012**:860213.
177. Tayel MY, Soliman E, El Baz WF, El Labaan A, Hamaad Y, Ahmed MH: **Registry of the clinical characteristics of spondyloarthritis in a cohort of Egyptian population**. *Rheumatol Int* 2012, **32**:2837-2842.
178. Roberts RL, Wallace MC, Jones GT, van Rij AM, Merriman TR, Harrison A, White D, Stamp LK, Ching D, Highton J, et al.: **Prevalence of HLA-B27 in the New Zealand population: effect of age and ethnicity**. *Arthritis Res Ther* 2013, **15**:R158.
179. Kirijas M, Mishevska-Perchinkova S, Karadzova-Stojanoska A, Efinska-Mladenovska O, Petlichkovski A, Trajkov D, Spiroski M: **Association between HLA-A, HLA-C and HLA-B genes and ankylosing spondylitis in macedonian population**. *Macedonian Journal of Medical Sciences* 2012, **5**:263-268.
180. Solmaz D, Akar S, Soysal O, Akkoc Y, Can G, Gerdan V, Birlik M, Onen F, Akkoc N: **Performance of different criteria sets for inflammatory back pain in patients with axial spondyloarthritis with and without radiographic sacroiliitis**. *Clin Rheumatol* 2014, **33**:1475-1479.
181. Bodur H, Ataman S, Bugdayci DS, Rezvani A, Nas K, Uzunca K, Emlakcioglu E, Karatepe AG, Durmus B, Sezgin M, et al.: **Description of the registry of patients with ankylosing spondylitis in Turkey: TRASD-IP**. *Rheumatol Int* 2012, **32**:169-176.
182. Acar M, Cora T, Tunc R, Acar H: **HLA-B27 subtypes in Turkish patients with ankylosing spondylitis and healthy controls**. *Rheumatol Int* 2012, **32**:3103-3105.
183. El Mouraghi I, Ouarour A, Ghozlani I, Collantes E, Solana R, El Maghraoui A: **Polymorphisms of HLA-A, -B, -Cw and DRB1 antigens in Moroccan patients with ankylosing spondylitis and a comparison of clinical features with frequencies of HLA-B*27**. *Tissue Antigens* 2015, **85**:108-116.
184. Atouf O, Benbouazza K, Brick C, Saoud B, Benseffaj N, Amine B, Hajjaj-Hassouni N, Essakalli M: **Distribution of HLA class I and II genes in ankylosing spondylitis patients from Morocco**. *Pathol Biol (Paris)* 2012, **60**:e80-83.
185. Mahfoudh N, Siala M, Rihl M, Kammoun A, Frikha F, Fourati H, Younes M, Gdoura R, Gaddour L, Hakim F, et al.: **Association and frequency of HLA-A, B and HLA-DR genes in south Tunisian patients with spondyloarthritis (SpA)**. *Clin Rheumatol* 2011, **30**:1069-1073.
186. Santos AM, Pena P, Avila M, Briceno I, Jaramillo C, Vargas-Alarcon G, Rueda JC, Saldarriaga EL, Angarita JI, Martinez-Rodriguez N, et al.: **Association of human leukocyte A, B, and DR antigens in Colombian patients with diagnosis of spondyloarthritis**. *Clin Rheumatol* 2017, **36**:953-958.

187. Brown MA, Kenna T, Wordsworth BP: **Genetics of ankylosing spondylitis--insights into pathogenesis**. *Nat Rev Rheumatol* 2016, **12**:81-91.
188. Tikly M, Njobvu P, McGill P: **Spondyloarthritis in sub-Saharan Africa**. *Curr Rheumatol Rep* 2014, **16**:421.
189. Rachid B, El Zorkany B, Youseif E, Tikly M: **Early diagnosis and treatment of ankylosing spondylitis in Africa and the Middle East**. *Clin Rheumatol* 2012, **31**:1633-1639.
190. Zhang S, Li Y, Deng X, Huang F: **Similarities and differences between spondyloarthritis in Asia and other parts of the world**. *Curr Opin Rheumatol* 2011, **23**:334-338.
191. Diaz-Pena R, Ouedraogo DD, Lopez-Vazquez A, Sawadogo SA, Lopez-Larrea C: **Ankylosing spondylitis in three Sub-Saharan populations: HLA-B*27 and HLA-B*14 contribution**. *Tissue Antigens* 2012, **80**:14-15.
192. Sakyi SA, Agyei-Frempong MT, Quansah RE: **HLA-B27 and its associated clinical and biochemical presentation among ghanaians with ankylosing spondylitis**. *Journal of Medical Sciences (Faisalabad)* 2012, **12**:161-167.
193. Khan MA: **Polymorphism of HLA-B27: 105 subtypes currently known**. *Curr Rheumatol Rep* 2013, **15**:362.
194. Khan MA: **An Update on the Genetic Polymorphism of HLA-B*27 With 213 Alleles Encompassing 160 Subtypes (and Still Counting)**. *Curr Rheumatol Rep* 2017, **19**:9.
195. Fallahi S, Mahmoudi M, Nicknam MH, Gharibdoost F, Farhadi E, Saei A, Nourijelyani K, Ahmadzadeh N, Jamshidi AR: **Effect of HLA-B*27 and its subtypes on clinical manifestations and severity of ankylosing spondylitis in Iranian patients**. *Iran J Allergy Asthma Immunol* 2013, **12**:321-330.
196. Reveille JD: **The genetic basis of spondyloarthritis**. *Ann Rheum Dis* 2011, **70 Suppl 1**:i44-50.
197. Johnsen SS, Bakland G, Nossent JC: **The distribution of HLA-B27 subtype in patients with ankylosing spondylitis in Northern Norway**. *Scand J Rheumatol* 2014, **43**:296-300.
198. Zhang L, Liu JL, Zhang YJ, Wang H: **Association between HLA-B*27 polymorphisms and ankylosing spondylitis in Han populations: a meta-analysis**. *Clin Exp Rheumatol* 2011, **29**:285-292.
199. Yang T, Duan Z, Wu S, Liu S, Zeng Z, Li G, Wang S, Fan D, Ye D, Xu S, et al.: **Association of HLA-B27 genetic polymorphisms with ankylosing spondylitis susceptibility worldwide: a meta-analysis**. *Mod Rheumatol* 2014, **24**:150-161.
200. Diaz-Pena R, Lopez-Vazquez A, Lopez-Larrea C: **Old and new HLA associations with ankylosing spondylitis**. *Tissue Antigens* 2012, **80**:205-213.
201. Zhang Z, Dai D, Yu K, Yuan F, Jin J, Ding L, Hao Y, Liang F, Liu N, Zhao X, et al.: **Association of HLA-B27 and ERAP1 with ankylosing spondylitis susceptibility in Beijing Han Chinese**. *Tissue Antigens* 2014, **83**:324-329.
202. Qi J, Li Q, Lin Z, Liao Z, Wei Q, Cao S, Rong J, Hu Z, Yang M, Zhang Y, et al.: **Higher risk of uveitis and dactylitis and older age of onset among ankylosing spondylitis patients with HLA-B*2705 than patients with HLA-B*2704 in the Chinese population**. *Tissue Antigens* 2013, **82**:380-386.
203. Kenna TJ, Brown MA: **Immunopathogenesis of ankylosing spondylitis**. *International Journal of Clinical Rheumatology* 2013, **8**:265-274.
204. Van Gaalen FA: **Does HLA-B*2706 protect against ankylosing spondylitis? A meta-analysis**. *Int J Rheum Dis* 2012, **15**:8-12.
205. Xiong J, Chen J, Tu J, Ye W, Zhang Z, Liu Q, Zhu X: **Association of HLA-B27 status and gender with sacroiliitis in patients with ankylosing spondylitis**. *Pak J Med Sci* 2014, **30**:22-27.
206. Chen HA, Chen CH, Liao HT, Lin YJ, Chen PC, Chen WS, Chien CC, Chou CT: **Clinical, functional, and radiographic differences among juvenile-onset, adult-onset, and late-onset ankylosing spondylitis**. *J Rheumatol* 2012, **39**:1013-1018.
207. Momenzadeh P, Mahmoudi M, Beigy M, Garshasbi M, Vodjdanian M, Farazmand A, Jamshidi AR: **Determination of IL1R2, ANTXR2, CARD9, and SNAPC4 single nucleotide polymorphisms in Iranian patients with ankylosing spondylitis**. *Rheumatology International* 2015:1-7.
208. Akkoc N, Yarkan H, Kenar G, Khan MA: **Ankylosing Spondylitis: HLA-B*27-Positive Versus HLA-B*27-Negative Disease**. *Curr Rheumatol Rep* 2017, **19**:26.

209. Ramiro S, Stolwijk C, van Tubergen A, van der Heijde D, Dougados M, van den Bosch F, Landewe R: **Evolution of radiographic damage in ankylosing spondylitis: a 12 year prospective follow-up of the OASIS study**. *Ann Rheum Dis* 2015, **74**:52-59.
210. Yang M, Xu M, Pan X, Hu Z, Li Q, Wei Y, Zhang Y, Rong J, Zhai J, He P, et al.: **Epidemiological comparison of clinical manifestations according to HLA-B*27 carrier status of Chinese ankylosing spondylitis patients**. *Tissue Antigens* 2013, **82**:338-343.
211. Sari I, Haroon N: **Editorial: Axial Spondyloarthritis: The Recurrence Plot Thickens**. *Arthritis and Rheumatology* 2016, **68**:2354-2356.
212. Stolwijk C, Boonen A, van Tubergen A, Reveille JD: **Epidemiology of spondyloarthritis**. *Rheum Dis Clin North Am* 2012, **38**:441-476.
213. Kim HW, Choe HR, Lee SB, Chang WI, Chae HJ, Moon JY, Kang J, Lee S, Song YW, Lee EY: **Phenotype difference between familial and sporadic ankylosing spondylitis in Korean patients**. *J Korean Med Sci* 2014, **29**:782-787.
214. Joshi R, Reveille JD, Brown MA, Weisman MH, Ward MM, Gensler LS, Wordsworth BP, Evans DM, Assassi S: **Is there a higher genetic load of susceptibility loci in familial ankylosing spondylitis?** *Arthritis Care Res (Hoboken)* 2012, **64**:780-784.
215. Shahlaee A, Mahmoudi M, Nicknam MH, Farhadi E, Fallahi S, Jamshidi AR: **Gender differences in Iranian patients with ankylosing spondylitis**. *Clin Rheumatol* 2015, **34**:285-293.
216. Arends S, Maas F, Wink F, Efde M, Bootsma H, Van der veer E, Brouwer E, Spoorenberg A: **Male and female patients with axial spondyloarthritis experience disease activity, physical function and quality of life differently: Results from the Groningen Leeuwarden Axial Spondyloarthritis cohort**. *Rheumatology (United Kingdom)* 2015, **54**:1333-1335.
217. Khan MA, Haroon M, Rosenbaum JT: **Acute Anterior Uveitis and Spondyloarthritis: More Than Meets the Eye**. *Current Rheumatology Reports* 2015, **17**.
218. Gunes A, Erkol Inal E, Tok L, Tok O: **Assessment of Corneal Parameters with Scheimpflug Imaging in Patients with Ankylosing Spondylitis**. *Seminars in Ophthalmology* 2015.
219. Popescu C, Trandafir M, Badica A, Morar F, Predeteanu D: **Ankylosing spondylitis functional and activity indices in clinical practice**. *J Med Life* 2014, **7**:78-83.
220. Reveille JD: **An update on the contribution of the MHC to AS susceptibility**. *Clin Rheumatol* 2014, **33**:749-757.
221. Kim TJ, Sung IH, Lee S, Joo KB, Choi JH, Park DJ, Park YW, Lee SS, Kim TH: **HLA-B27 homozygosity has no influence on radiographic damage in ankylosing spondylitis: Observation Study of Korean spondyloArthropathy Registry (OSKAR) data**. *Joint Bone Spine* 2013, **80**:488-491.
222. van Gaalen FA, Verduijn W, Roelen DL, Bohringer S, Huizinga TW, van der Heijde DM, Toes RE: **Epistasis between two HLA antigens defines a subset of individuals at a very high risk for ankylosing spondylitis**. *Ann Rheum Dis* 2013, **72**:974-978.
223. Atagunduz P, Aydin SZ, Bahadir C, Erer B, Direskeneli H: **Determinants of early radiographic progression in ankylosing spondylitis**. *J Rheumatol* 2010, **37**:2356-2361.
224. Mou Y, Zhang P, Li Q, Lin Z, Liao Z, Wei Q, Gu J: **Clinical Features in Juvenile-Onset Ankylosing Spondylitis Patients Carrying Different B27 Subtypes**. *Biomed Res Int* 2015, **2015**:594878.
225. Chavan H, Samant R, Deshpande A, Mankeshwar R: **Correlation of HLA B27 subtypes with clinical features of ankylosing spondylitis**. *Int J Rheum Dis* 2011, **14**:369-374.
226. Deodhar A, Mease PJ, Reveille JD, Curtis JR, Chen S, Malhotra K, Pangan AL: **Frequency of Axial Spondyloarthritis Diagnosis Among Patients Seen by US Rheumatologists for Evaluation of Chronic Back Pain**. *Arthritis Rheumatol* 2016, **68**:1669-1676.
227. Khan MA: **Ankylosing spondylitis and related spondyloarthropathies: the dramatic advances in the past decade**. *Rheumatology (Oxford)* 2011, **50**:637-639.
228. Garg N, van den Bosch F, Deodhar A: **The concept of spondyloarthritis: where are we now?** *Best Pract Res Clin Rheumatol* 2014, **28**:663-672.
229. Busquets-Pérez N, Marzo-Ortega H: **How important is early diagnosis of ankylosing spondylitis for therapy in clinical practice?** *International Journal of Clinical Rheumatology* 2012, **7**:575-578.
230. van den Berg R, van der Heijde DM: **How should we diagnose spondyloarthritis according to the ASAS classification criteria: a guide for practicing physicians**. *Pol Arch Med Wewn* 2010, **120**:452-457.

231. Salehi-Abari I, Khazaeli S, Khak M: **Early diagnosis of ankylosing spondylitis: an introduction to the newly designed Iran criteria for ankylosing spondylitis**. *Rheumatol Int* 2013, **33**:1303-1308.
232. Jung SH, Cho SM, Yim SH, Kim SH, Park HC, Cho ML, Shim SC, Kim TH, Park SH, Chung YJ: **Developing a Risk-scoring Model for Ankylosing Spondylitis Based on a Combination of HLA-B27, Single-nucleotide Polymorphism, and Copy Number Variant Markers**. *J Rheumatol* 2016, **43**:2136-2141.
233. Chow S, Haroon N: **A 24-year-old man with suspected sacroiliitis**. *CMAJ* 2015, **187**:124-125.
234. Poddubnyy D, Vahldiek J, Spiller I, Buss B, Listing J, Rudwaleit M, Sieper J: **Evaluation of 2 screening strategies for early identification of patients with axial spondyloarthritis in primary care**. *J Rheumatol* 2011, **38**:2452-2460.
235. Rudwaleit M, Sieper J: **Referral strategies for early diagnosis of axial spondyloarthritis**. *Nat Rev Rheumatol* 2012, **8**:262-268.
236. Sieper J, Srinivasan S, Zamani O, Mielants H, Choquette D, Pavelka K, Loft AG, Geher P, Danda D, Reitblat T, et al.: **Comparison of two referral strategies for diagnosis of axial spondyloarthritis: the Recognising and Diagnosing Ankylosing Spondylitis Reliably (RADAR) study**. *Ann Rheum Dis* 2013, **72**:1621-1627.
237. Seo MR, Baek HL, Yoon HH, Ryu HJ, Choi HJ, Baek HJ, Ko KP: **Delayed diagnosis is linked to worse outcomes and unfavourable treatment responses in patients with axial spondyloarthritis**. *Clinical Rheumatology* 2015, **34**:1397-1405.
238. Fallahi S, Jamshidi AR: **Diagnostic delay in ankylosing spondylitis: Related factors and prognostic outcomes**. *Archives of Rheumatology* 2016, **31**:24-30.