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EpHLA: An innovative and user-friendly software automating the HLAMatchmaker algorithm for antibody analysis

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ABSTRACT

The global challenge for solid organ transplantation programs is to distribute organs to the highly sensitized recipients. The purpose of this work is to describe and test the functionality of the EpHLA software, a program that automates the analysis of acceptable and unacceptable HLA epitopes on the basis of the HLAMatchmaker algorithm. HLAMatchmaker considers small configurations of polymorphic residues referred to as eplets as essential components of HLA-epitopes. Currently, the analyses require the creation of temporary files and the manual cut and paste of laboratory tests results between electronic spreadsheets, which is time-consuming and prone to administrative errors.

Results: The EpHLA software was developed in Object Pascal programming language and uses the HLAMatchmaker algorithm to generate histocompatibility reports. The automated generation of reports requires the integration of files containing the results of laboratory tests (HLA typing, anti-HLA antibody signature) and public data banks (NMDP, IMGT). The integration and the access to this data were accomplished by means of the framework called *eDAFramework*. The *eDAFramework* was developed in Object Pascal and PHP and it provides data access functionalities for software developed in these languages. The tool functionality was successfully tested in comparison to actual, manually derived reports of patients from a renal transplantation program with related donors.

Conclusions: We successfully developed software, which enables the automated definition of the epitope specificities of HLA antibodies. This new tool will benefit the management of recipient/donor pairs selection for highly sensitized patients.

1. Introduction

The interaction among HLA molecules and antibodies has been in the limelight among researchers and clinicians in the history of organ transplantation. Patel and Terasaki showed with lymphocytotoxicity cross-match tests [1] a correlation between donor-reactive antibodies and poor graft survival, and this made this test a mandatory pre-transplant evaluation [2]. Subsequently, issues were raised about the sensitivity and specificity of the complement dependent lymphocytotoxicity assays (CDC), and this led to the development of the solid phase assay methods (SPA) which are now used on a worldwide basis. Especially single allele panels have been useful to test for HLA antibodies [3]. This technique has also been used to predict cross-matches in sensitized candidates and to monitor the development of clinically relevant HLA antibodies post-transplant.

A new outlook of the HLA–antibody interaction in the transplantation context was reported when Rene Duquesnoy reasoned that the antibody interacts not with "HLA antigens", but with structurally defined epitopes called eplets, present in the HLA molecules. According to this hypothesis, different HLA molecules will be recognized by the same antibody if such HLA molecules have one or more eplets in common recognized by that antibody [4]. Characterizing eplet-specific

Abbreviations: SPA, Solid Phase Assay; AMM, Acceptable Mismatches; GUI, Graphical user interface; CDC, Complement-dependent Cytotoxicity; LIB, Immunogenetics and Molecular Biology Laboratory; UFPI, Federal University of Piauí; SSOPH, Sequence-specific Oligonucleotide Probe Hybridization; PRA, Panel of Reactive Antibodies; NMDP, National Marrow Donor Program; MFI, Median-Fluorescence Intensity; IMGT, the ImMunoGeneTics program; CREG, Cross Reactive Group.

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antibodies is useful to identify acceptable mismatches (AMM). In this sense, AMM are HLA antigens which differ from the patient's own HLA antigens, but they do not have antibody-eplets. Realizing that establishing AMM increases the transplantation chances in highly sensitized patients, Duquesnoy and collaborators developed HLA-Matchmaker, a donor–recipient compatibility algorithm based on eplets that may react with antibodies [5]. This algorithm, validated by the Eurotransplant group, increases the rate of transplantation among highly sensitized recipients with a shorter waiting time. In fact, every highly sensitized recipient entering the AMM Program has a 43% chance of receiving a transplant within 12 months, or 58% within 21 months. The follow-up of these recipients showed that the graft survival at two years is 87%, the same result as that observed for non-sensitized recipients transplanted in the same period [6]. These results, which were confirmed by other groups [7–9], point to AMM Program as an alternative for transplantation of highly sensitized recipients against HLA antigens.

Data Input for HLAMatchmaker algorithm is a set of data resulting from the screening for the presence of HLA antibodies in the recipient's serum (SPA Results). Data output from HLAMatchmaker is a set of eplets that permits an expert laboratory personnel working in the HLA field to identify AMM. Unfortunately, both input data into HLAMatchmaker and output data analyses are manually performed with labor-intensive Microsoft Excel programs, which limit applying the eplet - concept in the clinically oriented HLA laboratory. Currently, there is no software automating the input and output data analysis for HLA-Matchmaker. A computerized tool and a centralized relational database would reduce potential analyses errors, increasing reproducibility of histocompatibility studies, facilitating the data management and making data analysis less labor-intensive and more clinically applicable. The EpHLA software has been developed to carry out HLAMatchmaker in HLA laboratories that serve clinical transplant programs. It provides searches with a non-redundant and structured local database managed through a graphical user interface (GUI).

2. Objectives

The purpose of this work is to describe and test the functionality of the EpHLA software, a program that automates the analysis of acceptable and unacceptable HLA epitopes on the basis of the HLAMatchmaker algorithm.

3. Materials and methods

3.1. Execution

EpHLA is built in the Object Pascal programming language and uses an MS-Access (http://office.microsoft.com/pt-br/access/default.aspx) [10] or MySQL (http://www.mysql.com/) [11] database to store clinical and genetic data. In order to ease data integration between HLAMatchmaker, Solid Phase Assay (SPA) results and web repositories, we developed the easy Data Access framework (*eDAframework*). This framework was developed in Object Pascal (http://delphi.com/) [12] and PHP (Hypertext Preprocessor — http://www.php.net/) [13] programming languages and provides import, data access and export functionalities. The import functionality allows the importing of data from different file formats (FASTA, text files, comma separated values and Excel spreadsheet — http://office.microsoft.com/pt-br/excel/default.aspx [14]) to laboratory local databases, releasing them to access at only one repository. Such data can be accessed through eDAframework and used for processing through the EpHLA software. The results of this processing are exported as Excel spreadsheets using the export functionality.

The EpHLA software uses the HLAMatchmaker algorithm to find acceptable and unacceptable mismatches for HLA sensitized recipients. The input data to the HLAMatchmaker algorithm are: donor and recipient's HLA alleles, serum date, cutoff value and the SPA results. However, if high resolution HLA alleles are not available, allele frequencies databases can be queried in order to define the most likely allele for each case. The HLAMatchmaker algorithm works by comparing eplets found in donor and recipient's HLA molecules, generating a list of matches and mismatches for each other. The reports generated by EpHLA program allow laboratory personnel to divide potential donors into three different categories: (i) full HLA match; (ii) acceptable mismatches, and (iii) unacceptable mismatches. Note that if donor and recipient HLA molecules are identical, their eplets are identical too, and the transplant is acceptable. On the other hand, if organ donor/recipient HLA molecules are not identical, two cases are possible: (i) The recipient has preformed antibodies against donor eplets; (ii) The recipient does not have antibodies against donor eplets. In the first case, there is a higher risk associated with the transplantation, and in the second one, there is a lower risk [2, 15].

3.2. Using the system

The EpHLA program runs without complex setup procedures: the user has only to copy its files to drive C on a computer executing the Windows or MAC operational system (using a virtual machine). The EpHLA software consists of an executable program (EpHLA.exe), a relational database and auxiliary directories, as shown in the directory tree of Fig. 1, [A].

The EpHLA program's workflow consists of five steps: 1. Preparation of CSV files with the SPA results; 2. The processing of one or more CSV files; 3. The inclusion of the HLA alleles from recipient and donor; 4. Definition of cutoff value of SPA results; and 5. Generation of the Histocompatibility Map report.

Preparation of CSV files is related to transferring CSV files to the *input* directory of the EpHLA program's directory tree. The CSV files copied to the *input* directory are shown in the form Available *CSV files in directory* (Fig. 1, [B]). Using this form, one or more files can be selected and processed (workflow's second step). The EpHLA software uses information available in the HLAMatchmaker program's spreadsheets ([5] http://www.hlamatchmaker.net), including class of HLA and lot number of SPA kits (obtained from the manufacturer — Fig. 1, [C]). The result of the processing is available in the EpHLA — *Local repository* form. This form contains information on the recipient and his/her SPA results. Thus, one must access the *Local repository* form of the EpHLA software and type in the class I and class II HLA alleles of the recipient and donor.

The next step is to determine the cutoff value. The standard value of the EpHLA program is 500 of Median-Fluorescence Intensity (MFI). However, the laboratory personnel can define the value or alter to the suggested value in section *Calculated Cutoff*, according to Rene Duquesnoy [16] (Fig. 2). In the last step, the EpHLA program executes the HLAMatchmaker algorithm to generate the Histocompatibility Map report. During this step, the recipient's eplets of the self HLA molecules are removed from the histocompatibility analysis; the remaining eplets (non-self) are shown in the Histocompatibility Map report and classified by the EpHLA program as potentially or weakly immunogenic based on the adopted MFI cutoff value. All alleles of the panel whose MFI is lower than the cutoff established by the laboratory personnel will have its eplets classified as weakly immunogenic in all HLA molecules studied. These eplets are shown in blue. Otherwise, the eplet is considered potentially immunogenic and is typed black or red. A black eplet means that it is not the only eplet responsible for immunogenicity of the HLA molecule. On the other hand, a red eplet stands for a unique eplet responsible for immunogenicity in at least one HLA molecule for the tested serum whose MFI value is larger than the cutoff.

The Histocompatibility Map report from the EpHLA program contains two tabsheets: (i) Eplets Map and (ii) Eplet's Report. Eplets Map contains five predictable tabs groupings: Acceptable Mismatches, No