Guidelines for the Blood Transfusion Services

Chapter 17: Granulocyte immunology

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Chapter 17:

Granulocyte immunology

17.1: Reagent manufacture/reference preparations/cell panels

17.1.1: HNA typing reagents

There are several human neutrophil antigen (HNA) genotyping and phenotyping techniques. The latter are generally based on the use of polyclonal HNA alloantibodies obtained from immunised donors or patients or monoclonal antibodies. HNA typing techniques that do not require polyclonal antibodies derived from donors or patients are the techniques of choice.

HNA typing reagents prepared from human source material should comply with the guidelines in section 11.1.4.10.

An ‘Instructions for use’ sheet (package insert) should be prepared and supplied with antibody typing reagents, see section 11.1.4.12. Information in the instructions for use sheet should further indicate the immunoglobulin class of the antibodies and the presence of any other contaminating antibodies reactive by the recommended methods.

HNA typing reagents used in genomic DNA and polymerase chain reaction (PCR)-based techniques should comply with the guidelines in Chapter 14.

17.1.2: Composition of granulocyte cell panel for HNA antibody detection

It is recommended that laboratories make all reasonable efforts to include cells in their panel that will aid the detection and identification of clinically significant HNA antibodies. The panel should consist of granulocytes typed for HNA-1a, 1b, 1c, 2, 3a, 3b, 4a, 4bw, 5a and 5bw by validated HNA typing techniques. A minimum panel should include granulocytes that are homozygous for HNA-1a and HNA-1b and preferably be from Group O donors. The panel can be expanded to include granulocytes homozygous for other HNA as indicated by the results of laboratory testing.

HNA typing of a granulocyte panel donor should ideally be based on two concordant typing results performed on samples obtained on different occasions. Wherever possible, both phenotyping and genotyping should be performed for the above antigens.

17.1.3: The preparation of granulocytes/lymphocytes

Granulocytes and lymphocytes for use in serological investigations should be prepared with regard to the following criteria:

- Granulocytes/lymphocytes should be prepared from donors/patients within 24 hours of venesection. Precautions must be taken to minimise activation of granulocytes during isolation.
- Granulocyte/lymphocyte preparations should be essentially free from red cells that would otherwise interfere with the technique or its reading.
- The viability of isolated granulocytes should be sufficient as to not interfere in the technique used.

17.1.4: Selection of normal control sera

Normal control sera should be taken from untransfused male blood donors. The sera should be screened and found negative for granulocyte-reactive antibodies (e.g. clinically non-significant autoantibodies are occasionally detected in apheresis donors). An appropriate number of normal sera should be used, so that in any given assay a statistically relevant normal range can be determined.

17.1.5: Selection of positive control sera

At least one positive control should be included in each assay. The selection and number of positive control sera will depend on the technique and the HNA type of the granulocytes being used. In glycoprotein-specific assays, a positive control for each glycoprotein used should be included as a minimum. If different capture monoclonal antibodies are used, the positive control selected should be reactive with the monoclonal antibody selected.

17.1.6: Reference preparations

Sensitivity of techniques should be monitored on the basis of the inclusion of a ‘weak positive’ control. For anti-HNA-1a, the internal sensitivity control should be calibrated against the WHO International Reference Reagents for anti-HNA-1a (NIBSC code 09/284) when diluted as instructed by the manufacturer.

In-house sensitivity standards, with similar reaction strengths to the above reagent, should be prepared for other HNA antibodies.

17.1.7: Quality control schemes

Laboratories should take part in regular external quality control exercises such as the International Granulocyte Immunology Workshops for HNA antibody detection and for HNA genotyping. Effective mechanisms should be in place to correct poor performance in the quality scheme.

17.2: Nomenclature

The current nomenclature for HNA and corresponding antibodies is based on epitopes and allelic typing and this must be used for recording granulocyte-specific alloantigen and alloantibody specificities[1] (see Table 17.1).

Table 17.1 Current nomenclature for HNA alleles, epitopes and corresponding antibodies

System	Allele	Epitopes	Original name	Glycoprotein	CD

HNA-1	*FCGR3B*01*	HNA-1a	NA1	FcRIIIb	CD16
	*FCGR3B*02*	HNA-1b, HNA-1d	NA2	FcRIIIb	CD16
	*FCGR3B*03*	HNA-1b, HNA-1c	SH	FcRIIIb	CD16
	*FCGR3B*04*	HNA-1a		FcRIIIb	CD16
	*FCGR3B*05*	HNA-1bv		FcRIIIb	CD16
HNA-2	Unknown	HNA-2	NB1	GP56-64kDa	CD177
HNA-3	*SLC44A2*01*	HNA-3a	5b	CTL-2	
	*SLC44A2*02*	HNA-3b	5a	CTL-2	
	*SLC44A2*03*	HNA-3av		CTL-2	
HNA-4	*ITGAM*01*	HNA-4a	MART[a]	CD11/18	CD11b
	*ITGAM*02*	HNA-4bw		CD11/18	CD11b
HNA-5	*ITGAL*01*	HNA-5a	OND[a]	CD11/18	CD11a
	*ITGAL*01*	HNA-5bw		CD11/18	CD11a
v: indicates a variant epitope that may have different serological characteristics with some antisera.					
w: indicates a workshop designation – no antibodies against this epitope have currently been described.					

17.3: HNA typing methods

HNA types should be determined using antibody-based and/or DNA/PCR-based techniques that have been validated in the laboratory.

Polyclonal human anti-HNA antisera used in serological techniques should be well characterised. There is no requirement to use typing antisera that are ABO compatible with the granulocytes since the available evidence suggests that granulocytes either do not express Blood Group A or B antigens or do so very weakly. However, human HNA antisera may contain HLA class I antibodies that may confound results.

DNA based HNA typing techniques should be capable of distinguishing the different allelic forms described in Table 1 or, where ambiguities are recognised in distinguishing different alleles, these should be clearly identified in any reports.

17.4: HNA antibody detection methods

There are several techniques for the detection of HNA-reactive antibodies. These techniques

can be divided into non-specific (where intact granulocytes are used, e.g. granulocyte immunofluorescence test, granulocyte agglutination test) and specific assays (where glycoprotein capture, or purified glycoproteins or recombinant antigens are used, e.g. monoclonal antibody immobilisation of granulocyte antigen test). Laboratories should use tests with adequate sensitivity for the detection and identification of HNA-reactive antibodies. It is recommended that more than one technique is used to detect HNA-specific antibodies.

The combination of chosen technique(s) and the composition of the cell panel cells (if applicable) must ensure:

- The detection of clinically significant HNA-reactive alloantibodies to the antigens of the HNA-1, HNA-2, HNA-3, HNA-4 and HNA-5 systems.
- The detection and identification of HNA-reactive antibodies in samples containing a mixture of both HNA and HLAreactive antibodies, including antibodies to HNA-3 system antigens, which are expressed on both granulocytes and lymphocytes.
- The identification of the individual HNA specificities in samples containing mixtures of alloantibodies against several HNA antigens (e.g. masking of certain HNA specificities by composition of the panel).
- Techniques should be available to detect cytotoxic and non-cytotoxic anti-lymphocyte antibodies and thereby aid the distinction between granulocyte-specific, lymphocyte-reactive and HLA Class I and Class II antibodies.
- Assays for the detection of granulocyte antibodies , which utilise glycoproteins isolated from human cells, soluble recombinant antigens attached to a solid phase or recombinant cell lines expressing HNA should be used in parallel with established human granulocyte-based tests, either ‘in house’ or at a reference laboratory, while further data on the performance of these tests is gathered. An antibody specificity determined on the basis of reactivity with a single recombinant antigen or single isolated membrane glycoprotein should be viewed as indicative rather than definitive. Further work should be undertaken to confirm the antibody specificity using other sources of the implicated antigen. If the ‘indicative’ antibody specificity is confirmed by other techniques the original result can be used as supporting evidence to satisfy the requirements in 17.5.2. The existing advice that, wherever possible, a patient or donor with suspected HNA specific alloantibodies should either be genotyped to determine if they are negative for the allele encoding the implicated antigen or be phenotyped to ensure the absence of the antigen (17.5.2) should be applied.

Where granulocyte-specific antibodies are detected, which appear to have allo-specificity, but the specificity cannot be determined, the samples should be referred to a reference laboratory for further antibody specificity investigations. However, laboratories should make all reasonable efforts to screen against the widest possible range of HNA antigens.

17.4.1: Validation of laboratory kits

- Kits for the detection of HNA-reactive antibodies should be validated for sensitivity and specificity on a batch basis using a panel of clinically representative HNA antisera. It is recommended that the

sensitivity of HNA antibody detection should be monitored using a panel of antisera containing ‘weak’ reactive HNA antibodies (not obtained by dilution of strongly reactive HNA typing sera). A panel of sera shown to be inert for HNA and HLA antibodies should also be used.
- Kits for HNA typing should be validated for specificity on a batch basis using nine donors (three donors homozygous for each HNA allele together with three heterozygotes).

17.5: Donor testing

17.5.1: HNA typing

HNA typing of donors whose granulocytes may be transfused to support HNA-immunised recipients should, wherever possible, be typed twice using samples collected on different occasions. However, it may be necessary to issue HNA-selected products on the basis of a single or ‘unconfirmed’ type.

17.5.2: Investigation of HNA antibodies

HNA antibody specificities should only be assigned when the sample investigated has been tested and a minimum of three positive and three negative reactions obtained with a single technique or a minimum of two positive and two negative reactions with two techniques. A report identifying the antibody can be issued at this stage. A donor with an HNA alloantibody should receive an HNA antibody card and an information leaflet, wherever this is available. However, before an HNA antibody card and information leaflet is issued, the donor should be typed (on one occasion but ideally by two methods) and found negative for that antigen.

17.5.3: Investigation of female donors to reduce the incidence of TRALI

Many transfusion services have introduced screening for HLA or HLA and HNA antibodies to reduce the incidence of transfusion-related acute lung injury (TRALI). An initial screen for HLA antibodies may be followed by a screen for HNA antibodies to further reduce the potential incidence of TRALI. Female blood donors should be investigated for HNA antibodies following the guidelines for donor investigation, except that there is only a requirement to test for IgG antibodies. The screening techniques used should, as a minimum, enable detection of HNA-1a, -1b, -2 and -3a antibodies which are known to be implicated in causing TRALI.

HLA antibodies should be investigated using the guidelines set out in section 16.6.

17.6: Patient testing

17.6.1: HNA typing

Patients should be typed for HNA following the guidelines for donor HNA typing. A provisional type can be issued on the basis of a phenotype/genotype performed on one occasion. However, it is recommended that, if possible, a second typing technique be used on the first occasion of testing, especially where quality exercises or routine practice have revealed technical problems in typing for particular polymorphisms.

17.6.2: Investigation of HNA antibodies

Patients should be investigated for HNA antibodies following the guidelines for donor investigation. The investigation of neonatal alloimmune neutropenia (NAIN) should include HNA typing of the parents and affected baby(ies) as this will help identify any potential HNA incompatibilities and can be used to direct antibody screening if the father and baby both have a low frequency HNA that is absent in the mother. Cases with a clinical diagnosis of possible NAIN and a negative HNA antibody screen against common HNA should be investigated for the presence of antibodies against low-frequency or 'private' antigens. An effective approach is to use granulocytes from the child's father as an additional panel cell (paternal granulocytes should be HNA typed as a 'patient sample'). Alternatively, laboratories may refer such cases to a reference laboratory.

In the investigation of TRALI, implicated donor samples should be investigated for the presence of both HNA and HLA Class I and Class II antibodies (see also section 16.6). There is usually no requirement to investigate the patient's serum for HNA or HLA antibodies, but if this is necessary both pre- and post-transfusion samples (where available) should be investigated. Where antibody specificities are identified, the donor and patient should be typed to determine the presence or absence of the cognate antigen. If required, a crossmatch may be performed between the implicated donor serum samples and granulocytes /lymphocytes from the patient to determine the clinical relevance of any antibodies and the presence of any low-frequency antibodies. When 'pooled' platelet products are implicated in a case of TRALI, consideration should also be given to the possibility of the formation of inter-donor immune complexes. In such cases, all the donors who contributed to the pool should also be HNA and HLA typed. In a small proportion of TRALI cases, patient antibodies may react with infused donor cells/antigens and it may be necessary to incubate the patient's serum with granulocytes/lymphocytes from the donor.

Crossmatch studies in both suspected NAIN and TRALI cases require that the granulocytes/lymphocytes are isolated from the patient's blood samples within 24 hours of venesection.

A patient or donor with HNA alloantibodies should receive an HNA antibody card and an information leaflet wherever this is available.

17.6.3: Controls for direct tests for granulocyte bound immunoglobulins

Anticoagulated blood samples, less than 24 hours old, from a sufficient number of different normal donors to give a statistically valid normal range, should be used as control samples for the determination of granulocyte-bound immunoglobulins.

17.7: References

1. Flesch BK, Curtis BR, de Haas M, Lucas G, Sachs UJ (2016). Update on the nomenclature of human neutrophil antigens and alleles. *Transfusion*, 56, 1477–1479.