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SPECIAL REPORT

# SCT for severe autoimmune diseases: consensus guidelines of the European Society for Blood and Marrow Transplantation for immune monitoring and biobanking

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Over the past 15 years, SCT has emerged as a promising treatment option for patients with severe autoimmune diseases (ADs). Mechanistic studies recently provided the proof-of-concept that restoration of immunological tolerance can be achieved by haematopoietic SCT in chronic autoimmunity through eradication of the pathologic, immunologic memory and profound reconfiguration of the immune system, that is, immune 'resetting'. Nevertheless, a number of areas remain unresolved and warrant further investigation to refine our understanding of the underlying mechanisms of action and to optimize clinical SCT protocols. Due to the low number of patients transplanted in each centre, it is essential to adequately collect and analyse biological samples in a larger cohort of patients under standardized conditions. The European society for blood and marrow transplantation Autoimmune Diseases and Immunobiology Working Parties have, therefore, undertaken a joint initiative to develop and implement guidelines for 'good laboratory practice' in relation to procurement, processing, storage and analysis of biological specimens for immune reconstitution studies in AD patients before, during and after SCT. The aim of this document is to provide practical recommendations for biobanking of samples and laboratory immune monitoring in patients with ADs undergoing SCT, both for routine supportive care purposes and investigational studies.

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## INTRODUCTION

### Background

Autoimmune diseases (ADs) are a heterogeneous group of diseases affecting 8–10% of the population. Therapeutic immunosuppression and novel biological therapies can suppress or attenuate the inflammatory process as long they are applied, but cannot switch off the underlying mechanisms inducing therapy-free remission, that is, cure. Although effective in most cases, chronic immunosuppression is associated with the reduction in quality-of-life, cumulative toxicity and increased risk of cardiovascular disease, and represents a considerable socioeconomic challenge. For patients with major organ involvement and therapy-resistant disease, high-dose immunosuppression followed by haematopoietic SCT has been used since 1995[1] worldwide and was shown to induce treatment-free, long-term remissions in several ADs.[2–6] More recently, the use of MSCs has shown promising results in chronic GVHD[7] and ADs,[8] and common procedures for immune monitoring and biobanking will be applied for these various types of SCT in AD patients.

### The concept of SCT for ADs

On the basis of experimental data from animal models,[9,10] haematopoietic SCT for ADs is applied with the goal of eradicating the autoreactive immunologic memory and to regenerate a naive and self-tolerant immune system from haematopoietic precursors. It has remained unclear for a long time whether clinical remissions observed after haematopoietic SCT were the result of prolonged immunosuppression or the outcome of a true reconfiguration of

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**Table 1.** Minimum requirements for immune monitoring before and after SCT in AD patients

| *Biological sample* | *Recommended analyses* | *Methods* | *Time-point of analysis* |
|---|---|---|---|
| Serum | Total Ig levels (IgG, IgA, IgM) | ELISA | At baseline before the mobilization and SCT, at (3), 6, (9), 12 months after SCT and biannually thereafter |
| | Autoantibody titres (in Ag-mediated ADs) | ELISA, immunofluorescence | |
| PBMCs | Expression analysis of CD45, CD3, CD4, CD8, CD19, CD56 and CD14 | Cytometry | |

Brackets indicate optional time-points for analysis.

**Table 2.** Recommendation for storage of biological samples and their potential exploitation for immunologic investigations before and after SCT in AD patients

| *Biological sample* | *Potential analyses* | *Time-point of cryopreservation* |
|---|---|---|
| Serum | Proteomic profiling, cytokines, growth factors, autoantibodies, circulating microRNAs | At baseline before the mobilization and SCT, at (3), 6, (9), 12 months after SCT and biannually thereafter |
| PBMCs | Cytometric profiling | |
| DNA | Genome-wide association studies, epigenetic analysis, TRECs & KRECs | |
| RNA | Gene expression profiles, microRNA arrays | At baseline before the mobilization and SCT and yearly after SCT |

Abbreviations: KRECs=κ-deleting recombination excision circles; TRECs=T-cell receptor excision circles. Brackets indicate optional time-points for analysis.

the immune system. A number of recent immunological studies have provided the proof-of-concept that a chronic autoreactive immune system can indeed be 'reset' into a naive and self-tolerant immune system. These data include the regeneration of naive B cells,[5,11] thymic reactivation,[5,12–14] the emergence of a polyclonal TCR repertoire[5,12,14] and restoration of $Foxp3^+$ regulatory T-cell (Treg) levels.[15,16] Although interest in MSC usage was originally raised by their potential capacity to differentiate into different cell lineages, recent work showing their immunological properties has led to a revized concept, envisioning their utilization for immunomodulatory purposes. Their clinically relevant immunomodulatory potential has been demonstrated in clinical trials for GVHD[7] and in various types of ADs.[8]

### Rationale for the development of guidelines for immune monitoring and biobanking in AD patients after SCT

Early clinical guidelines and recommendations[17] for SCT in ADs were recently updated.[18] Meanwhile, activity in the field is expanding with results from randomized phase II/III studies in major disease indications, such as systemic sclerosis (ASTIS).[2] In parallel, clinical trials of MSC transplantation have been growing in number.[8] Although the mechanisms of action of SCT in ADs have been partially elucidated, important issues remain unresolved from both clinical and scientific points of view. These include the variability associated with type of AD, patient age, continued use of immunosuppression and the effect of different transplantation techniques, for example, variable conditioning regimens and graft manipulation technology. The low number of patients transplanted at each centre requires multicentre studies under standardized conditions. The European Society for Blood and Marrow Transplantation Autoimmune Diseases and Immunobiology Working Parties have, therefore, undertaken a combined initiative to develop guidelines for harmonized 'good laboratory practice' in biobanking and immune monitoring before and after SCT in patients with ADs. These guidelines do not replace established standard operating procedures in individual laboratories, and national regulatory requirements should always prevail. However, they aim to support the establishment of an international biobanking infrastructure and common testing protocols, thereby facilitating collaborative and comparative research studies in the field of stem cell therapies for ADs.

## GENERAL RECOMMENDATIONS

### Clinical practice guidelines

In AD patients considered for SCT, referral should be made to a centre with appropriate inter-disciplinary interaction between haematological and AD specialists. Such centres should have JACIE accreditation or equivalent[18] and should provide programmes for long-term follow-up, quarterly for the first year and biannually follow-up consultation, thereafter, ideally with dual review by both haematologists and disease specialists with assessment of disease-specific activity scores. Where possible, laboratories should participate in internal and external quality assurance schemes for flow cytometry, molecular genetics and immunological markers. National and/or EU regulations should be followed in relation to biobanking of cells and tissues.

### Guidelines for basic laboratory immune analyses and biological sample storage

A minimum set of laboratory analyses is recommended for immune monitoring in patients before and at certain time-points after SCT by all contributing centres (Table 1). These include full blood count analysis on freshly isolated PBMCs, allowing enumeration of $CD4^+$ and $CD8^+$ T cells, $CD19^+$ B cells, $CD3^-$/$CD56^+$ natural killer cells, $CD3^+$/$CD56^+$ natural killer-like T cells and $CD14^+$ monocytes. In addition, monitoring of total Ig levels and analysis of serum autoantibody titres in Ab-mediated ADs are recommended at baseline before the mobilization and SCT, quarterly for the first year and biannually thereafter. In addition to the mentioned basic laboratory immune analyses, storage of biological samples is recommended for extended immune monitoring at later time-points. A list of biological samples recommended for storage and potential immunologic investigations is provided (Table 2). To promote harmonized sample handling across different sites, standardized protocols for sample

**Table 3.** Panels for flow cytometric analyses

| *Panel* | *Analysis of markers* | *Time-point of analysis* |
|---|---|---|
| T-cell differentiation | CD4, CD8, CD45RA, CD31, CCR7 CD28, CD27, CD57 | At baseline before the mobilization and SCT, at (3), 6, (9), 12 months after SCT and yearly thereafter |
| T-cell receptor subsets and activation | CD4, CD8, TCRab, TCRgd, CD69, CD38, CD45RA, CD25 | |
| Regulatory T cells | CD4, CD8, Foxp3, CD25, CD127, CD45RA, Ki-67 | |
| B cells | CD19, IgD, IgM, CD27, CD38, CD24 | |
| Plasma cells | CD19, CD27, CD20, CD38, CD138, HLA-DR | |
| DCs | CD45, CD11c, HLA-DR, BDCA-2, CD86 | |
| MSC | CD45, CD34, CD80, CD86, CD73, CD90, CD105, CD271 | |
| Master transcription factors | CD3, CD4, T-bet, GATA-3, RORγt, Bcl-6 | |

Brackets indicate optional time-points for analysis.

collection, processing and storage have been developed and are provided in the Supplementary Appendix.

### Expert centre recommendations for extended immune monitoring

For centres with expertize in flow cytometry and adequate immune monitoring core facilities, it is recommended to analyse a broader set of biomarkers in patients before and after SCT. For immunocytometric analysis, eight panels were defined, dedicated to fine cytometric analyses of the different subsets of cells within the major cellular populations (Table 3). Comparison between centres may be possible given a preliminary calibration of all flow cytometers with the same rainbow fluorescent particles. For in-depth investigation of the T- and B-cell lymphopoiesis, it is recommended to analyse T-cell receptor excision circles[19] and κ-deleting recombination excision circles,[20] which can be measured simultaneously using duplex real-time PCR.[21,22] Furthermore, TCR repertoire analysis of peripheral blood T cells is suggested with Vbeta analysis and flow cytometry, spectratyping or high-throughput sequencing.[23] Providing that efficient eradication of the autoreactive memory is a prerequisite for favourable long-term responses, it is suggested to analyse the frequency of autoantigen-specific T cells before and after SCT where possible, for example, islet-Ag-specific T cells in type 1 diabetes.[24] Here, tetramer staining[24,25] or magnetic pre-enrichment of $CD154^+$ T cells provides techniques for high-resolution analysis of Ag-reactive T cells directly from peripheral blood.[26] In addition, gene expression profiling of either whole blood or lymphocyte subsets, using microarray technology, at defined time-points before and after SCT should be considered (Table 2).

### Implications of immune monitoring for clinical practice: recommendations for infection prophylaxis and re-vaccination

Current Autoimmune Diseases Working Party guidelines already recommend that all patients receive *Pneumocystis jiroveci*, herpes and antifungal prophylaxis and are monitored for CMV and EBV (ideally by PCR) for infection (primary or reactivation) for at least 3 months after transplant, with active surveillance thereafter up to 2 years after SCT according to local practice.[18] At that stage, $CD4^+$ cell counts should be repeated and, if still below 200 cells/μL, prophylaxis or quarterly monitoring should be continued. If the patient suffers from recurrent or a life-threatening infection despite neutrophil recovery and has Ig or Ig subclass deficiency, i.v. Ig substitution should be considered after weighing up the benefits, risks and costs. Given the previous and often ongoing administration of immunosuppressive drugs following SCT, measurement of specific Ab levels is recommended before and after re-vaccination when performed according to published generic guidelines[27] to confirm protection and administer booster vaccination in patients with inadequate response.

## DISEASE-SPECIFIC RECOMMENDATIONS

### Multiple sclerosis and neuromyelitis optica

Multiple sclerosis (MS) is the most common acquired demyelinating disease of the central nervous system. MS is the result of an autoimmune inflammatory attack initiated by T and B cells and directed against components of central nervous system myelin. Axonal damage becomes prominent in secondary progressive disease and is the main cause of irreversible disability. HLA genes have a primary role in genetic susceptibility to MS, with a predominant role from HLA-DRB1*15 in the majority of populations.[28,29] Recent GWAS identified multiple non-MHC loci affecting the risk of developing MS.[30] The majority of these genes are immune related, for example, IL-2 and IL-7 receptors, CD6, CD58, IRF8, and TNFRSF1A genes. The most commonly used rating scale to grade neurological disability in patients with MS is the Expanded Disability Status Scale.[31] Historically considered a variant of MS, neuromyelitis optica is recognized as a distinct disease entity, whose hallmark is immune-mediated inflammation of optic nerves and the spinal cord caused by autoantibodies to the water channel aquaporin 4.[32,33] Besides being pathogenic, aquaporin 4 Abs are used as a marker of 'minimal residual AD' after SCT.[34] In addition to the general guidelines and immune monitoring panels described above, specific recommendations for MS and neuromyelitis optica are provided (Table 4).

### Systemic sclerosis

Systemic sclerosis (SSc) is a rare AD (prevalence 5–50 per 100 000) characterized by early endothelial vascular damage with activation of the immune response and enhanced collagen synthesis. Ag stimulation and genetic susceptibility contribute to autoimmunity, with consequent T- and B-cell activation, and fibroblast activation by pro-fibrotic cytokines, that is, transforming growth factor-β and connective tissue growth factor. Early T-cell infiltrates in skin and pulmonary tissue, autoantibody production by plasma cells, notably, anti-centromere and anti-topoisomerase-I and the presence of macrophages or altered endothelial cells promote inflammation and fibrosis. Several genetic associations have been observed between HLA types and autoantibody profiles, and genome-wide screening studies identified specific nucleotide polymorphisms in relevant genes related to SSc.[35] More recently, use of microarray technology showed significant differences of gene patterns in skin biopsies from patients with diffuse and limited SSc, which also differed from normal controls.[36] In this context, a few years ago, the EUSTAR biobanking group

**Table 4.** Disease-specific recommendations for immune monitoring before and after SCT in AD patients

| *Autoimmune disease* | *Biological sample* | *Recommended analyses* | *Methods* | *Time-point of analyses* |
|---|---|---|---|---|
| Multiple sclerosis and neuromyelitis optica | Serum | Autoantibodies: Anti-aquaporin 4 (only in NMO patients) | ELISA, immunofluorescence | At baseline before the mobilization and SCT, at (3), 6, (9), 12 months after SCT and biannually thereafter |
| | PBMCs | Abs against viruses: JCV | ELISA | At baseline before the mobilization and SCT, at (3), 6, (9), 12 months after SCT and yearly thereafter |
| | | MAIT cells: CD3, CD4, CD8, CD161, TCRVa7.2, CCR6 and IL-18R | FACS | |
| | | Tregs: CD3, CD4, CD8, CD103, CD25, CD62L, Foxp3 and CD127 | FACS | |
| | Cerebrospinal fluid | Oligoclonal bands, IgG and IgM (also in matched serum), differential cell count, storage of cell pellet RNA | Immunofixation, electrophoresis | At baseline before the mobilization and SCT, and 12 months following SCT |
| Systemic sclerosis | Serum | Complement levels: C3, C4 | ELISA | At baseline before the mobilization and SCT, at (3), 6, (9), 12 months after SCT and biannually thereafter |
| | | Autoantibodiy titres: Anti-centromere, anti-topoisomerase-I and anti-polymerase | ELISA | |
| | Skin biopsies | Phenotype of leukocytes and fibroblasts | FACS, immunohistology | At baseline before the mobilization or SCT, and 12 months following SCT |
| | | Gene expression analysis Fibroblasts | Microarray Fibroblast culture | |
| Systemic lupus erythematosus | Serum | Complement levels: C3 and C4 | ELISA | At baseline before the mobilization and SCT, at (3), 6, (9), 12 months after SCT and biannually thereafter |
| | | Autoantibody titres: Anti-dsDNA, Abs to extractable nuclear Ags, anti-Cardiolipin Abs | ELISA, immunofluorescence | |
| | | IFNα or IFN response proteins, e.g., IP-10 | ELISA | |
| | PBMCs | Tregs: CD4, Foxp3, CD25, Helios and Ki-67 | FACS | At baseline before the mobilization and SCT, at (3), 6, (9), 12 months after SCT and yearly thereafter |
| | | Siglec-1 expression on $CD14^+$ monocytes | FACS | |
| | | Low-density granulocytes: CD11b, CD15, CD16, CD33, CD86 and HLA-DR | FACS | |

**Table. 4.** (Continued)

| *Autoimmune disease* | *Biological sample* | *Recommended analyses* | *Methods* | *Time-point of analyses* |
|---|---|---|---|---|
| Crohn's disease | Serum | Cytokines: IL-17A, IL-22, IL-6, IFNγ | ELISA | At baseline before the mobilization and SCT, at (3), 6, (9), 12 months after SCT and biannually thereafter |
| | | Matrix metalloproteinase 9 | ELISA | |
| | Faeces | Calprotectin | ELISA | |
| | PBMCs | Activated T cells: CD4, CD38, CD45RA, HLA-DR and Ki-67 | FACS | At baseline before the mobilization and SCT, at (3), 6, (9), 12 months after SCT and yearly thereafter |
| | | Th17 T cells: CD4, IL-17 and IL-22 | FACS after polyclonal stimulation *in vitro* | |
| | Intestinal biopsies | Phenotype of leukocytes | FACS, immunohistology | At baseline before the mobilization or SCT and 12 months after SCT |
| | | Gene expression analysis | Microarray | |
| Type 1 diabetes | Serum | Autoantibodies: anti-GAD65, IA-2 antibody titres | ELISA | At baseline before the mobilization and SCT, at (3), 6, (9), 12 months after SCT and biannually thereafter |
| | | HbA1c | ELISA | |
| | | C-peptide secretion (mixed meal tolerance test) | ELISA | |
| | Other | Insulin need/kg | | |
| | PBMCs | Islet autoreactive T cells: $CD4^+$ and $CD8^+$ T cells specific for GAD65, insulin (B9-23), and IA-2 (709-736) peptides | FACS after *in vitro* stimulation | At baseline before the mobilization and SCT, at (3), 6, (9), 12 months after SCT |
| Rheumatoid arthritis and juvenile ideopathic arthritis | Serum | Autoantibodies: rheumatoid factor (RF), anti-cyclic *citrullinated peptide* (anti-CCP) | ELISA | At baseline before the mobilization and SCT, at (3), 6, (9), 12 months after SCT and biannually thereafter |
| | PBMCs | TCR Vβ family analysis | FACS | At baseline before the mobilization and SCT and yearly after SCT |
| | | Telomere length analysis | Quantitative PCR | |
| | Synovial fluid mononuclear cells | Lymphocyte number and phenotype | FACS, immunohistology, microscopic synovitis score | At baseline before the mobilization or SCT and 12 months following SCT (if applicable) |

Abbreviation: NMO=neuromyelitis optica. Brackets indicate optional time-points for analysis.

developed guidelines for collection, storage and distribution of SSc biospecimens (www.eustar.org). In addition to documenting a minimal essential data set, acquisition and storage of blood samples, and skin biopsies from patients with SSc after SCT are strongly encouraged in expert centres (Table 4).

### Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a rare chronic AD (prevalence 20–150 cases per 100 000) with heterogeneous clinical manifestations.[37] It is characterized by the generation of Abs directed against a variety of autoantigens, including nuclear and cytoplasmic Ags, and by complement activation.[37] Autoreactive plasma cells are key players in the induction and perturbation of immunopathology in SLE, and short-lived (HLA-DR$^{high}$) plasmablasts are readily detectable in the peripheral blood of patients with active disease.[38] Another hallmark of SLE is the upregulation of IFN-regulated gene transcripts.[39] IFNα and its response proteins IP-10 and Siglec-1 are established markers for monitoring disease activity in SLE.[40] Circulating Foxp3$^+$ Tregs are expanded in SLE, with Helios-expressing Tregs being the most prominent Treg subset, which correlates with disease activity.[41] Disease activity is most commonly measured by SLE disease activity index that comprises relevant clinical and laboratory values.[42] On the basis of these disease-specific features, recommendations for immune monitoring in SLE are provided (Table 4).

### Crohn's disease

Crohn's disease is a chronic inflammatory bowel disease characterized by recurring episodes of inflammation of the gastrointestinal tract. It is thought to arise by dysregulated mucosal immune responses to the gut flora in genetically susceptible individuals.[43] GWAS and meta-analyses have identified 140 susceptibility loci to Crohn's disease in Caucasians, but their heritability is not fully explained.[44] Disease activity is monitored by symptom-based scores, most commonly the Harvey–Bradshaw index[45] and the Inflammatory Bowel Disease Questionnaire.[46] Recent studies revealed an altered intestinal microbial and peripheral blood T-cell phenotype, in particular involvement of Th17 cells and IL-21/IL-22-producing CD4$^+$ T cells in Crohn's disease.[47,48] In addition, several biomarkers were shown to be important in assessment of disease, most notably serum cytokines (IL-22), metalloproteinase 9 and faecal calprotectin.[49] In addition to the general recommendations for immune monitoring, specific parameters of the immune system may be important in Crohn's disease (Table 4).

### Type 1 diabetes

Type 1 diabetes mellitus is an AD caused by autoreactive CD4$^+$ and CD8$^+$ T cells against insulin-producing islet cells.[50] Although the current standard treatment is insulin replacement therapy, several clinical trials for haematopoietic SCT have demonstrated long-term, insulin- and drug-free remissions in new-onset type 1 diabetes mellitus.[4,51] Following SCT, metabolic end points such as C-peptide secretion, HbA1c level and daily insulin need should be monitored. Immunological end points can give better information on the efficacy of the immune intervention. Although no correlation with disease activity has been described between several islet-specific Abs involved in type 1 diabetes mellitus (ICA-512/IA-2, GAD65),[50,52] their prognostic and predictive value in the context of SCT remains to be determined. Monitoring T-cell responses after SCT may be more relevant. Here, tetramer-staining techniques allow testing T-cell responses to HLA-A2-restricted insulin B10, pre-pro-insulin, islet Ag, GAD65 and pre-pro islet amyloid polypeptide.[24,25] We recommend also considering the publication of the T-cell workshop initiative of the Immunology of Diabetes Society regarding guidelines on how to handle biological samples in clinical type 1 diabetes mellitus trials.[53,54]

### Rheumatoid arthritis and juvenile idiopathic arthritis

Rheumatoid arthritis (RA), affecting ~1% of the population, is characterized by chronic joint inflammation, autoantibody production and variable degrees of bone and cartilage erosion.[55] Disease activity can be measured using the Disease Activity Scores and Clinical Disease Activity Index.[56] Immunopathology includes break of tolerance and accumulation of immune effector cells including macrophages and osteoclasts, DCs, B and T cells, especially Th17 subsets. Reduced T-cell receptor excision circles and shortened telomeres result in a contracted TCR repertoire in both naive and memory T cells.[57,58] Haematopoietic SCT was applied as a salvage therapy in severely affected patients in the 'pre-biologic era'. Although remission occurred in the majority of cases, recurrence was common, irrespective of CD34$^+$ graft selection.[59,60] In JIA patients undergoing Auto-SCT, restoration of initially reduced Foxp3$^+$ Treg levels was observed with change in autoreactive T cells from a pro-inflammatory (IFN-gamma and T-bet high) to a tolerant phenotype (IL-10 and GATA-3 high) after SCT.[15] Disease-specific recommendations for immune monitoring in RA/JIA are provided (Table 4).

## CONCLUSIONS

Although activity in the field of SCT for ADs is rapidly expanding, the interpretation of results obtained from immune monitoring of clinical trials is often limited due to the heterogeneity of methods that are used for sample processing and analysis. With the guidelines provided here, we aim to define 'good laboratory practice' in handling of biological samples and to harmonize methods for preparation, storage and analysis of biological specimens for immune reconstitution studies. The implementation and dissemination of these guidelines aim to support the establishment of an international biobanking infrastructure and common testing protocols. Adequate reporting and connection between the individual centres exploiting these data will foster collaborative and comparative research studies throughout Europe for better patient care and to refine our understanding of the underlying mechanisms of action that may directly translate into optimized protocols for SCT in ADs.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies this paper on Bone Marrow Transplantation website (http://www.nature.com/bmt)