In this issue: Clinical flow cytometry in 2019

The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

Flow cytometry in the diagnosis and follow up of human primary immunodeficiencies

Ulrich Salzer[1,2], Ulrich Sack[3], Ilka Fuchs[2,4]

[1] *Department of Rheumatology & Clinical Immunology, Medical Center - University of Freiburg, Faculty of Medicine, University of Freiburg, Germany*

[2] *Centre of Chronic Immunodeficiency, Medical Center - University of Freiburg, Faculty of Medicine, University of Freiburg, Germany*

[3] *Medical Faculty, Institute of Clinical Immunology, University of Leipzig, Leipzig, Germany*

[4] *Institute for Immunodeficiency, Medical Center - University of Freiburg, Faculty of Medicine University of Freiburg, Germany*

ARTICLE INFO

Corresponding author:
Ulrich Salzer, MD
Center for Chronic Immunodeficiency (CCI) and
Department of Rheumatology
and Clinical Immunology
Medical Center - University of Freiburg
Hugstetterstr. 55
79106 Freiburg im Breisgau
Germany
Phone/Fax: +49 761 27035390
E-mail: ulrich.salzer@uniklinik-freiburg.de

Key words:
flow cytometry, primary immunodeficiency, diagnostics

ABSTRACT

Primary immunodeficiencies (PID) comprise a group of more than 300 mostly monogenetic disorders of the immune system leading to infection susceptibility and a variety of associated clinical and immunological complications. In a majority of these disorders the absence, disproportions or dysfunction of leucocyte subpopulations or of proteins expressed by these cells are observed. These distinctive features are studied by multicolour flow cytometry and the results are used for diagnosis, follow up, classification and therapy monitoring in patients with PIDs. Although a definite diagnosis almost always relies on genetic analysis in PIDs, the results of flow cytometric diagnostics are pivotal in the initial diagnostic assessment of suspected PID patients and often guide the treating physician to a more selective and efficient genetic diagnostic procedure, even in the era of next generation sequencing technology. Furthermore, phenotypic and functional flow cytometry tests allow

to validate novel genetic variants and the mapping of complex disturbances of the immune system in individual patients in a personalized manner. In this review we give an overview on phenotypic, functional as well as disease/protein specific flow cytometric assays in the diagnosis of PID and highlight diagnostic strategies and specialties for several selected PIDs by way of example.

❖❖❖❖❖

INTRODUCTION

The last report of the International Union of Immunological Societies (IUIS) lists 354 inborn errors of immunity resulting in various forms of primary immunodeficiencies (PID), which are subdivided into nine categories:

1. Severe and combined immunodeficiencies (SCID)
2. Combined immunodeficiencies with syndromic features
3. Predominantly antibody deficiencies
4. Immunodysregulatory disorders
5. Defects affecting phagocytes
6. Defects of innate immunity
7. Autoinflammatory disorders
8. Complement deficiencies
9. Phenocopies of PIDs (1,2)

Although PIDs are generally considered as very rare disorders individually, they form a clinically relevant cohort as a group. The major clinical symptom in the majority of PIDs is infection susceptibility, which ranges from specific immunodeficiency to a single pathogen to broad immune failure. However some PIDs are syndromic disorders and many PIDs show additional clinical manifestations caused by immune dysregulation, including autoimmunity, lymphoproliferation, granulomatous inflammation and malignancy disposition. In addition, there is considerable phenotypic variability in many of the monogenetic PID traits and untreated or progressive PID disease causes sequelae, secondary changes and end organ damage.

Finally, secondary immunodeficiency caused by non-immunological disorders, environmental factors and immunosuppressive or ablative therapeutic interventions have to be considered especially in adult patients with manifesting symptoms suspicious for PID. Since most of severe PIDs manifest with first symptoms soon after birth, new born screening programs have recently been successfully established in several countries to detect these forms of PIDs at the earliest time point as possible (see article by Wolf J et al in this issue).

In summary, the complex clinical and immunological presentations of the various PIDs require sensitive and specific diagnostic tests. Flow cytometry emerged as a method of choice for the study of PIDs, since it allows the fast and reliable analysis of almost all branches of the immune system on a single cell level.

The basic clinical and laboratory evaluation of a patient with suspected PID should include a careful clinical history, paying special attention to the family history and the symptoms and features mentioned above, a complete differential blood count, serum immunoglobulin levels, a global complement function test and specific antibody titers for vaccine antigens (e.g. tetanus toxoid).

The individual flow cytometric diagnostic testing will depend on the clinical presentation of the patient and the results of basic laboratory tests. If a defect of adaptive immunity is suspected then usually a basic lymphocyte phenotyping will be performed followed by some more specific testing (e.g. extended phenotype of B-cells in patients with antibody deficiency). The range of applications ranges from phenotypic assays investigating the numbers and proportions of immune cells, functional analysis of

Table 1 Examples of basic and extended phenotypic assays, disease specific surface and intracellular protein analysis and functional assays to study PIDs*

Basic phenotypic analysis		
Test	**Cell populations**	**Indications**
Lymphocyte subpopulations	CD4+ T-cells, CD8+ T-cells, B cells, NK cells	Basic screening for PID, SCID
T-cell subpopulations	CD4+CD45RA+ naïve T-cells, HLA DR+ activated T-cells, CD8 effector cells, γ/δ T-cells, α/β double negative T-cells, regulatory T-cells	SCID, CVID, CID, ALPS
B-cell subpopulations	IgD+CD27- naïve B-cells, IgD+CD27+ non-switched memory, IgD-CD27+ switched memory, transitional B-cells, plasmablasts, CD21low B-cells	primary antibody deficiency, CVID, CID
Extended phenotypic analysis		
Test	**Cell populations**	**Indications**
dendritic cell subpopulations	CD123+ plasmacytoid dendritic cells, CD11c+ myeloid dendritic cells	GATA2 deficiency
regulatory T-cells	CD4+CD25+ FoxP3+ regulatory T-cells	IPEX syndrome
recent thymic emigrants	CD4+CD45RA+CD31+ T-cells	SCID, DGS
TCR repertoire analysis	T-cell Vβ chain variant expression on CD4 and CD8 T-cells	SCID, CID
Functional assays to study PIDs		
Test	**Cell populations**	**Indications**
oxidative burst assay	granulocytes	chronic granulomatous disease, inflammatory bowel disease
T-cell proliferation	CD4+ and CD8+ T-cells after stimulation with PHA, anti-CD3, anti-CD3 and anti-CD28	SCID, CVID, CID

NK cell degranulation	CD107a expression on stimulated or resting NK cells	familial hemophagocytic lymphohistiocytosis
IL-17/IFNγ production	PMA/Ionomycin stimulated T-cells	chronic mucocutaneous candidiasis, Hyper IgE syndrome
Disease specific surface and intracellular protein analysis		
Test	**Cell populations**	**Indications**
BTK	monocytes	X-linked agammaglobulinemia
WASp	lymphocyte subsets	Wiskott Aldrich syndrome
CD40L	activated T-cells	X-linked Hyper IgM syndrome
SAP	lymphocyte subsets	X-linked lymphoproliferative disorder type 1
XIAP	lymphocyte subsets	X-linked lymphoproliferative disorder type 2
Perforin	NK cells	FHL type 2

** ALPS: autoimmune lymphoproliferative syndrome; BTK: bruton tyrosine kinase; CID: combined immunodeficiency; CVID: common variable immunodeficiency; DGS: DiGeorge Syndrome; FHL: familial hemophagocytic lymphohistiocytosis; IPEX: Immune dysregulation, polyendocrinopathy, enteropathy, X linked; SAP: SLAM-associated protein; SCID: severe combined immunodeficiency; TCR: T-cell receptor; WASp: Wiskott Aldrich syndrome protein.*

cellular processes (e.g. proliferation, cytokine secretion, cytotoxicity) to the direct analysis of potentially mutated proteins in disease specific assays (Table 1). Usually these techniques are combined to allow the study of dynamic processes in specific cell subpopulations (e.g. degranulation of NK lymphocytes) or protein expression in specific cell populations after stimulation like e.g. CD40L expression in activated T-cells (see Table 1).

The setup and performance of these diagnostic assays at high and reproducible quality requires a high level of expertise from the laboratory. Reference values for phenotypic analysis of immune cells need to be age adapted and ideally should be determined for each tested parameter and setup individually. Especially for functional tests, in house reference values need to be determined and checked by appropriate tests on a regular basis. Since defined biological control materials for most of the assays will not be commercially available, the parallel testing of healthy controls for each diagnostic procedure is highly recommended. If available, standardized reagents and procedures should be used and followed.

Test results should be reported in context of the clinical presentation and a clear and direct communication between the laboratory staff and clinicians is often very important for the correct interpretation of the results.

To illustrate diagnostic strategies and possibilities of flow cytometry in the diagnostics of PID we will briefly discuss four different clinical scenarios within the field of PID:

1. X-linked (Bruton´s) agammaglobulinemia: a monogenetic PID with a limited clinical and immunological phenotype and some relevant differential diagnosis;
2. Common variable immunodeficiency disorders: a heterogeneous group of patients with primary antibody deficiency without known genetic defect but a clinically and immunologically highly diverse phenotype;
3. GATA2 haploinsufficiency: a monogenetic but clinically diverse trait affecting the cellular phenotype of different hematopoietic cell lineages, resulting in a complex immunopathology and diverse syndromic features;
4. Hemophagocytic lymphohistiocytosis (HLH), an often hyperacute and life threatening condition, in which flow cytometry is a fast and reliable diagnostic tool, which can assist in differentiating diverse primary and secondary causes.

Figure 1A **Basic lymphocyte subset analysis of an XLA deficient patient and a healthy control showing absent CD19+ B-cells (upper right panel)**

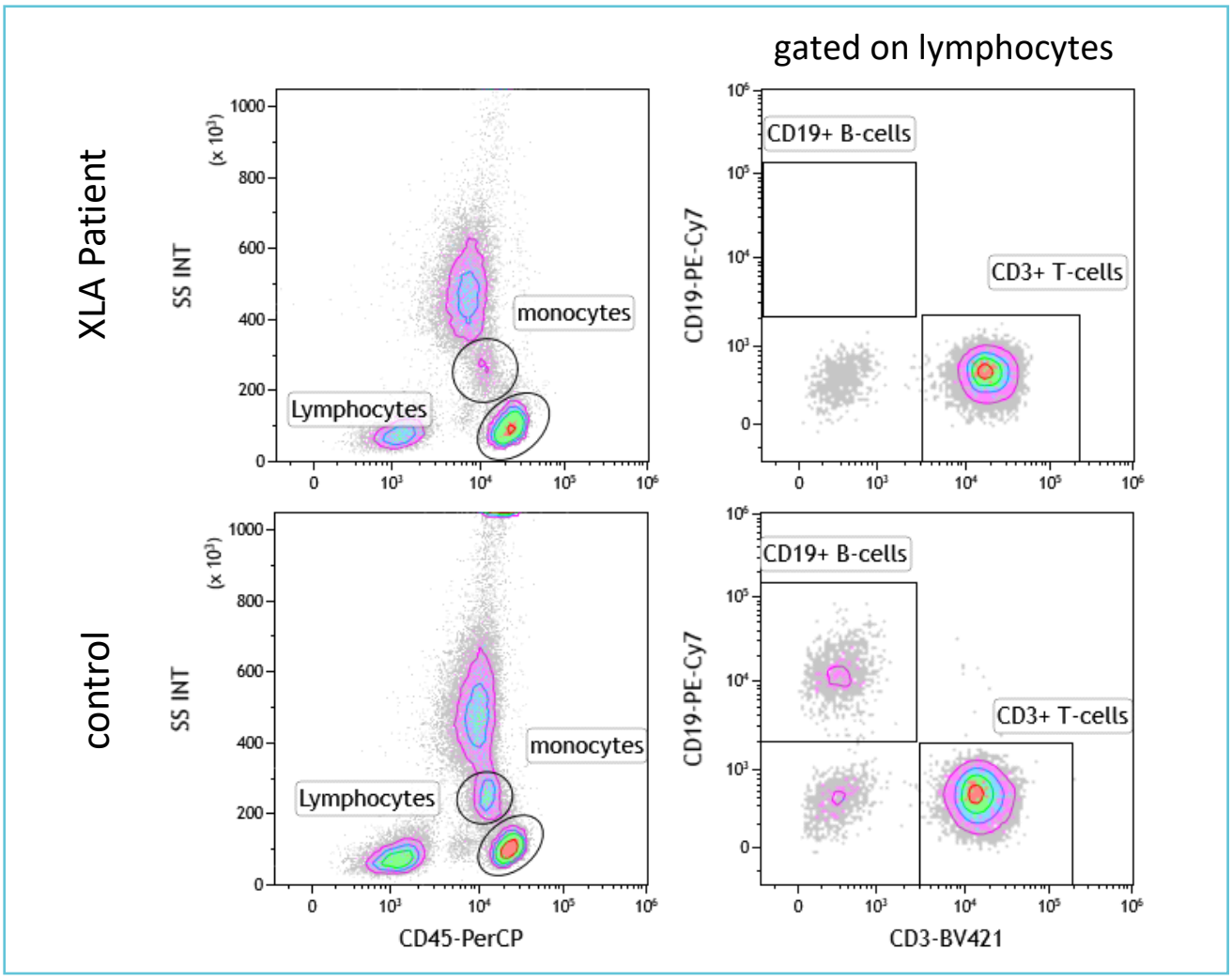
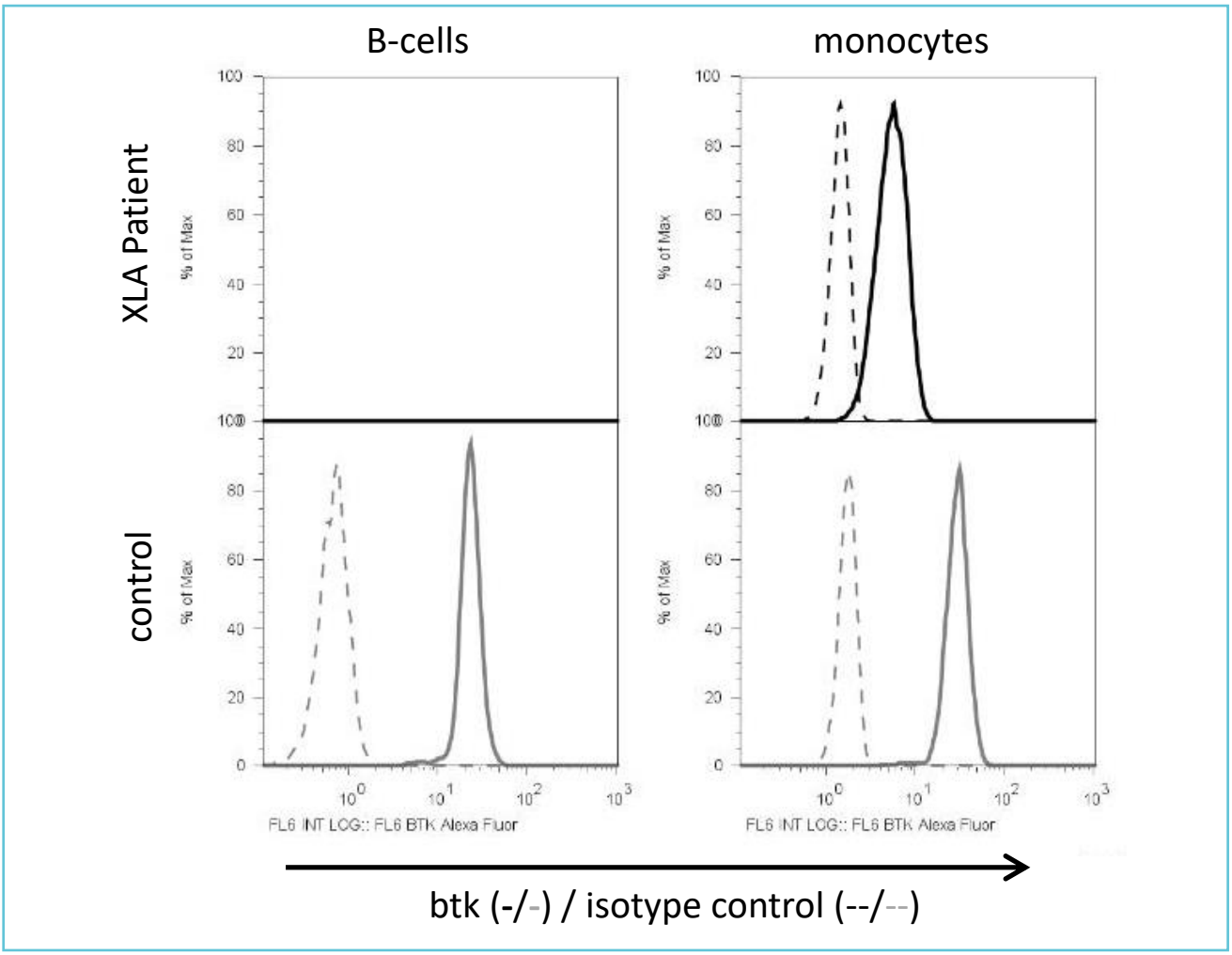


Figure 1B Reduced intracellular BTK expression (solid lines) versus the isotype control (dashed line) analyzed in monocytes (right panels) and B-cells (left panels) of an XLA patient a healthy control



X-LINKED AGAMMAGLOBULINEMIA (XLA)

The pairing of absent or very low cell B-cells and immunoglobulins is summarized in the group of agammaglobulinemias. 80% of affected children are male and most of these suffer from X-linked (Bruton´s) Agammaglobulinemia (XLA).

XLA was first described by OC Bruton in 1952, usually manifests in boys within the first two to five years of life and has a frequency of 1:1.000.000 live births (3,4). XLA is caused by mutations in the *btk* gene encoding for the Bruton Tyrosine Kinase (BTK) on the X-chromosome (5,6).

In developing B-cells in the bone marrow, BTK is important for signalling of the pre B-cell receptor and mutations found in XLA patients generally lead to a developmental block, resulting in severely impaired bone marrow output of B-cells (7).

Typically the patients develop bacterial infections of the respiratory tract, when maternally transferred antibody levels vanish after the sixth month of life (4). Total immunoglobulin levels are typically below 1 g/l but residual amounts of IgG, IgA and IgM may be present especially

in those XLA patients diagnosed after the age of five years (4).

Total lymphocyte numbers are usually normal and flow cytometric analysis of basic lymphocyte subpopulations (T, B, NK) reveals a normal T-cell and NK cell count, but B-cells are usually not detectable or below 1% of lymphocytes (see Table 1; Figure 1A).

In patients with suspected XLA BTK protein expression can be investigated by flow cytometry after intracellular staining in monocytes (8), which also express high levels of BTK and are present in sufficient numbers in patients with XLA (Figure 1B).

Most of the known *btk* mutations impair or abrogate BTK protein expression (9). However normal BTK protein levels do not exclude XLA and in cases where the clinical suspicion is high genetic analysis should be performed. Phosphorylation of BTK Y223 can be studied after pervanadate stimulation (10), providing a method to study the pathogenic relevance of uncertain novel mutations. In female and male patient with a normal *btk* gene autosomal recessive forms of agammaglobulinemias should be considered as differential diagnosis (11).

As these deficiencies affect the pre B-cell receptor complex and lead to characteristic cellular blocks in early B-cell development they could be easily identified by flow cytometry but require a bone marrow sample for analysis and thus are preferably unravelled by genetic analysis.

COMMON VARIABLE IMMUNODEFICIENCY DISORDERS (CVID)

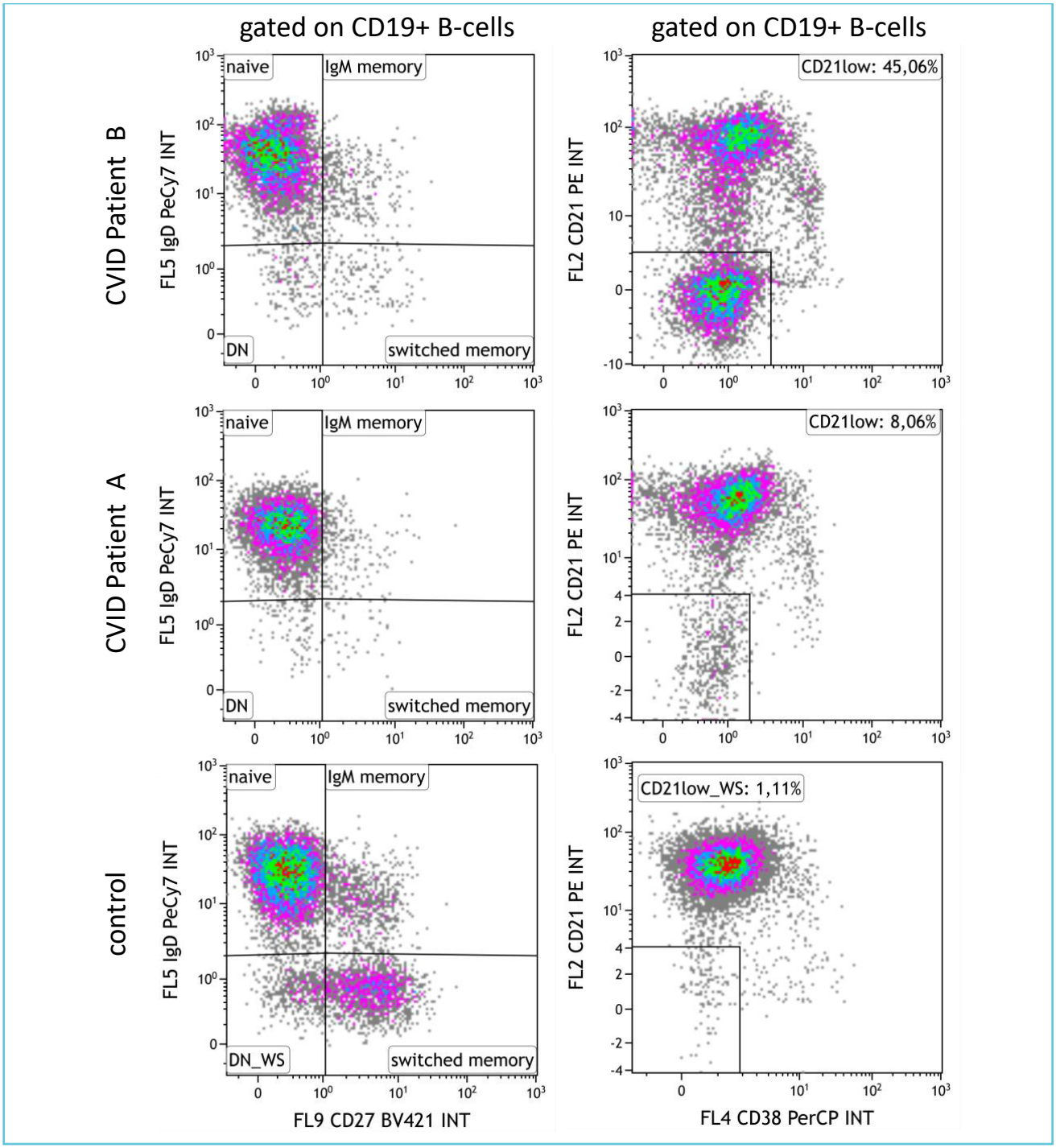
Common variable immunodeficiency disorders comprise the largest group of PID patients in adulthood. It is characterized by hypogammaglobulinemia, recurrent bacterial respiratory tract infections and several associated diseases or sequelae like autoimmune cytopenias, benign lymphoproliferation, granulomatous inflammation, and predisposition for certain malignancies and structural lung disorders.

Unlike most of the other primary immunodeficiencies, which manifest usually in the first year or decade of life, are mostly familial and have a defined monogenetic cause, CVID patients typically are adolescents or young adults when first symptoms occur and usually the cases are sporadic without a family history. As a diagnosis of exclusion CVID serves as a “drop box” for antibody deficiency syndromes of all kind that could not be attributed to any other known PID or other disease state manifesting primarily with hypogammaglobulinemia. Within the past decade it has been recognized that the initial 1999 PAGID / ESID criteria for the definition of CVID (12) need refinement and precision to better harmonize the CVID cohort and avoid misdiagnosis of CVID in patients, who actually suffer from different disorders requiring different care settings and therapy. Interestingly all new proposed diagnostic criteria include now some flow cytometric testing (13–15).

In relation the study of other PIDs, flow cytometric analysis has a special significance in the study of CVID and has contributed vitally to our understanding of these disorders. The cellular hallmark of CVID are severely reduced numbers of switched memory IgD-CD27+ B-cells and plasmablasts (see figure 2), which are observed in a majority of CVID patients (16–18). In subgroups of CVID patients additional B-cell disturbances like expansion of so called CD21low B-cells (see figure 2) or transitional B-cells are observed (17,19). Thus B-cell phenotyping by flow cytometry emerged as a useful tool for pathophysiological studies and the classification of the syndrome (17,20,21).

These studies showed several important significant relations between individual B-cell abnormalities and clinical complications, such as

Figure 2 **B-cell subpopulation analysis in two CVID patients and one healthy control showing naïve, IgM (or non-switched) memory and switched memory B-cells by anti IgD versus anti-CD27 staining (left panels) and CD21low B-cells by anti-CD21 versus anti CD38 staining (right panels). Both patients have reduced memory B-cell subsets and CVID patient B shows an expansion of CD21low B-cells.**



lymphoproliferation, granulomatous inflammation or autoimmune disease (17,20,21).

The early recognition of the development of such complications in individual CVID patients is mandatory, since they have an important impact on the morbidity and mortality (22). However, the B-cell phenotypes so far seem to have limitations as prospective biomarkers in individual CVID patients, likely because changes such as CD21low B-cell or transitional B-cell expansions are not CVID specific and may be only secondary to infections, lymphoproliferation, inflammation or autoimmunity (23–25). Besides B-cells, flow cytometric studies of the T-cell system have been widely used in CVID.

The recent novel definitions of CVID recommend a careful exclusion of severe T-cell deficiencies in patients with suspected CVID to avoid misdiagnosis in patients with LOCID (late on severe combined immunodeficiency) (26) or other forms of combined immunodeficiencies.

The revised criteria for the ESID registry require therefore a T-cell count of >200 / µl with an amount of at least 10% naïve CD4+CD45RA+ T-cells present in adults and/or a normal T-cell proliferation (13). However, the moderate reductions of naïve CD4+CD45RA+ T-cells found in a significant numbers of CVID patients have also been implicated as an alternative way of classification of disease associated pathologies in CVID (27).

In certain cases, flow cytometric screening or targeted analysis for changes in specific cell populations or cellular proteins has been successful to reveal single gene defects, such as ICOS deficiency, CD19 deficiency and BAFF-R deficiency (28–30).

However, these approaches cannot be recommended as routine diagnostics as these monogenetic defects are very rare and not all mutations will result in reduced protein expression and / or complete absence of a specific cell population.

GATA2 DEFICIENCY

In 2010 and 2011 two groups reported two similar novel PIDs, MONOmac syndrome (**mono**cytopenia and **m**ycobacterium **a**vium **c**omplex infections) and DCML (**d**endritic **c**ell, **m**onocyte, B and NK **l**ymphocyte) deficiency (31,32), characterized by autosomal dominant inheritance, certain cellular deficiencies, a variable and diverse susceptibility to infections and a predisposition to myeloid leukemia and infection associated cancers.

Subsequently, heterozygous mutations in the hematopoietic transcription factor *GATA2* have been identified as the genetic cause of the two syndromes (33,34) and several other conditions such as Emberger syndrome (sensorineuronal deafness and primary lymphedema with a predisposition for myelodysplastic syndrome or AML) and familial myelodysplastic syndrome or AML (35,36).

Missense mutations in the zinc-finger 2 domain or deleterious mutations of *GATA2* prevail, leading to functional or genetic GATA2 haploinsufficiency, which is required for hematopoietic stem cell (HSC) homeostasis (37).

In consequence GATA2 deficiency leads to depletion of HSC and especially lymphoid and myeloid precursors. Extrahematopoietic manifestations like thrombotic events, lymphedema or deafness are likely explained by the additional functions of GATA2 in vascular endothelia (38,39). The cellular phenotypes of GATA2 deficiency were studied in larger cohorts of patients and correlated with disease severity (40,41). Although each of the phenotypes is not specific to GATA2 deficiency, the joined appearance of these features is supportive in diagnosis. In particular the combination of monocytopenia, B- and NK cell deficiency (Figure 3A) together with low dendritic cell numbers (Figure 3B) should raise suspicion for GATA2 deficiency in patients with compatible clinical presentations.

HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS (HLH)

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening hyperinflammatory syndrome caused by different inherited and secondary conditions (42).

Primary HLH can be subdivided into the group of familial hemophagocytic lymphohistiocytosis (FHL). These are

- Perforin deficiency, FHL2;
- Munc13-4 deficiency, FHL3;
- Syntaxin 11 deficiency, FHL4 and
- Munc18-2 deficiency FHL-5

and several other monogenetic PIDs (among them are Chediak Higashi Syndrome (CHS), Griscelli syndrome type 2, X-linked lymphoproliferative syndrome type 1 and 2 (XLP1 and XLP2) and others) (43–50).

Secondary HLH occurs in association with viral infections, lymphoma, autoimmune disease, after hematopoietic stem cell transplantation and drug hypersensitivity.

Clinically both primary and secondary HLH may be triggered by viral infections and present with persistent fever, splenomegaly and bi- or trilinear cytopenias and show elevated levels of triglycerides, ferritin and soluble IL-2 receptor in serum (42).

Figure 3A **Basic lymphocyte subset analysis of a GATA2 deficient patient and a healthy control showing absent monocytes (upper left panel) and reduced CD19+ B cells (upper middle panel) and CD16+CD56+ NK cells (upper right panel)**

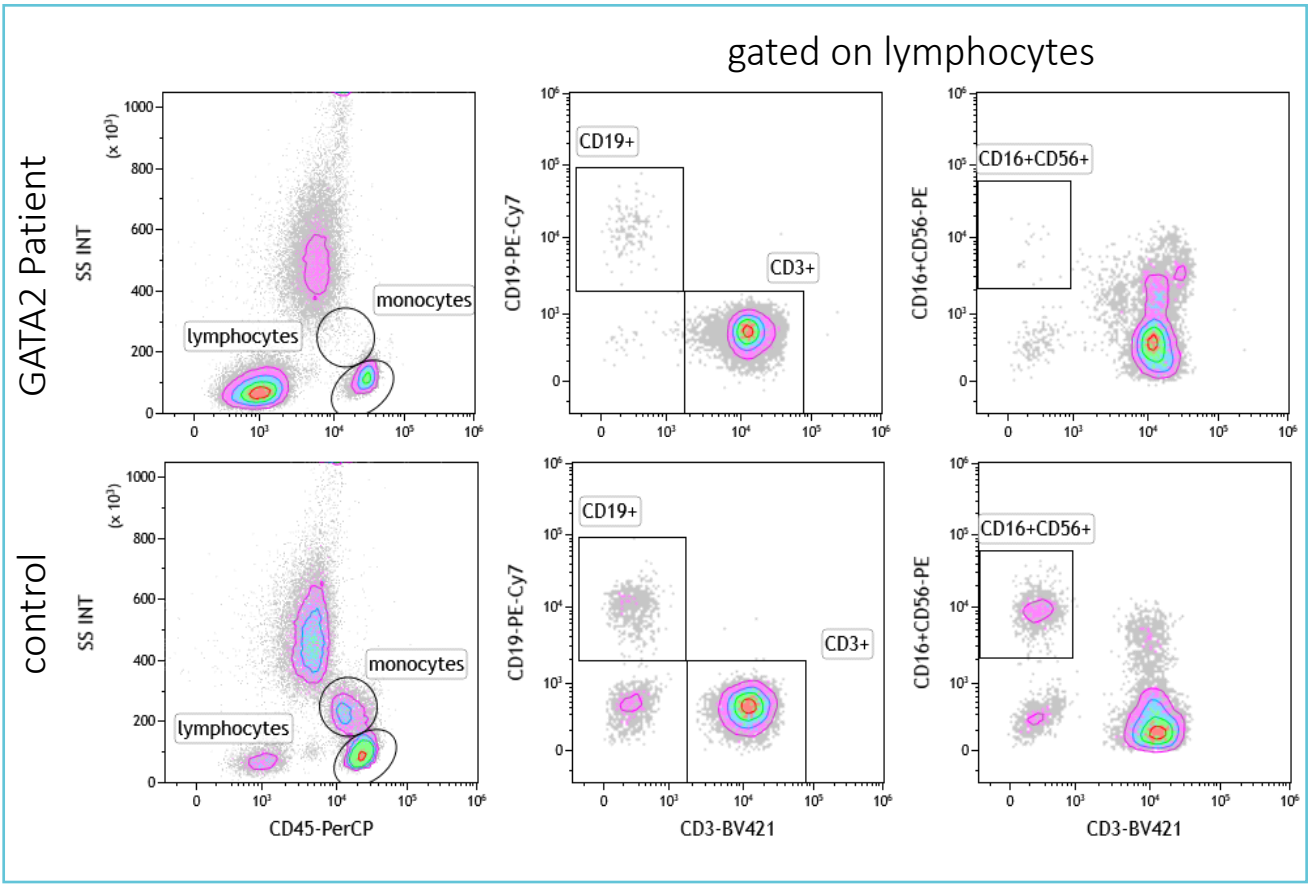
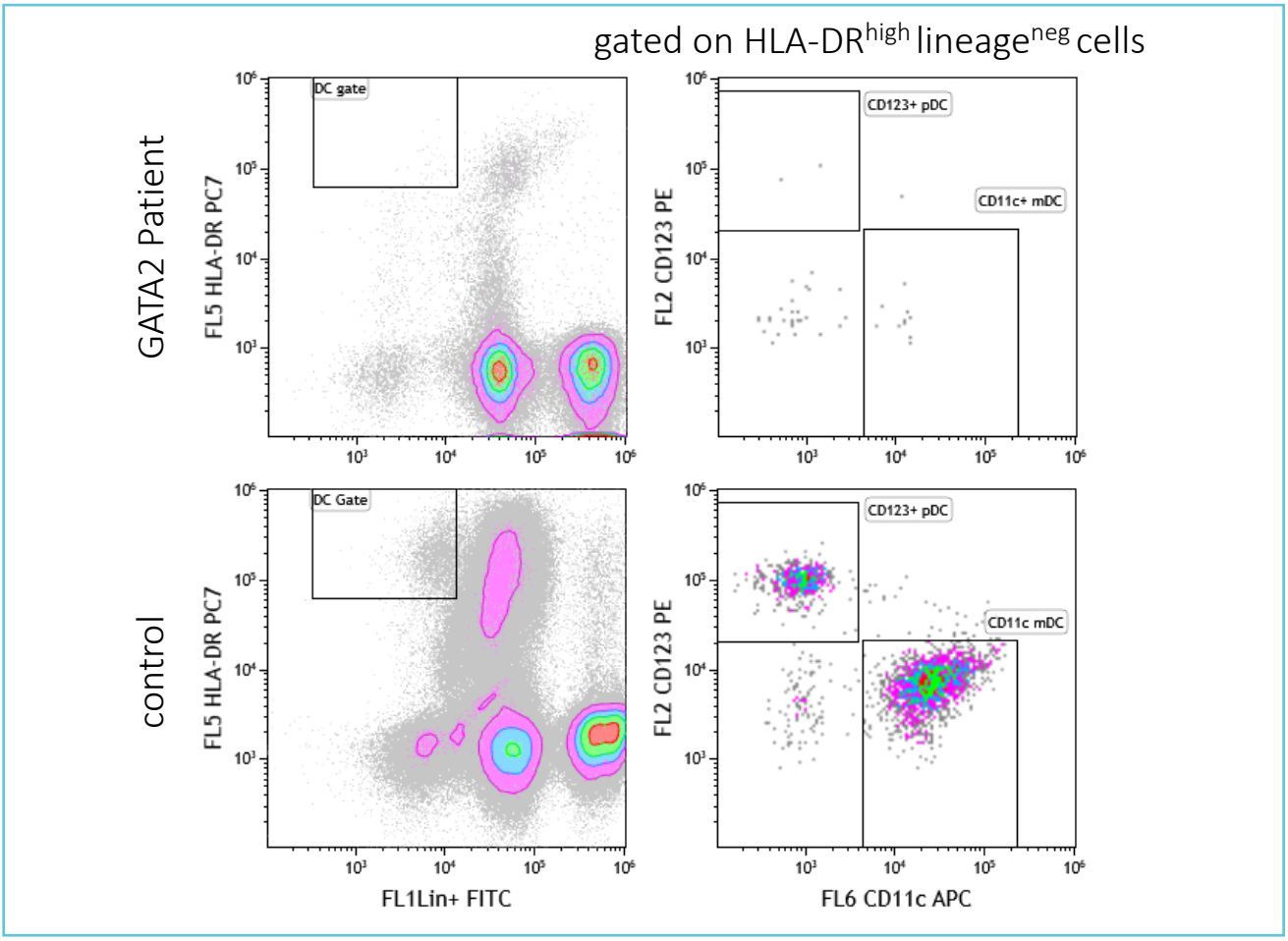


Figure 3B **Analysis of dendritic cell subsets in a GATA2 deficient patient and a healthy control revealing both severely reduced CD123+ lymphoid and CD11c+ myeloid dendritic cells (upper right panel)**



Primary and especially the subgroup of FHL usually manifests early in life whereas secondary HLH may occur at any age. Because the majority of FHL patients require hematopoietic stem cell transplantation, their rapid identification is critical.

FHL is caused by defects in the cytotoxic machinery of T-cells and NK cells and the deficiency of perforin (FHL2) is the prototypic form and the most common in FHL (43).

All other forms of FHL and the closely related disorders like CHS show defects in resting and activated NK cell degranulation, which can be detected by flow cytometric analysis of the cytotoxic granule associated marker CD107a. In XLP1 and XLP2 there is no apparent defect of cytotoxicity (51) but the patients develop HLH triggered by uncontrolled EBV infection due to deficiencies in the intracellular SAP and XIAP proteins (49,50).

A stratified flow cytometric work-up is very helpful in distinguishing the various forms of primary HLH from secondary HLH (51,52). Perforin, and in male patients also SAP and XIAP protein expression are analysed by intracellular flow cytometry (53,54) (Figure 4A).

If Perforin, SAP or XIAP protein expression is abnormal targeted genetic analysis of the respective

encoding genes should be performed. Otherwise degranulation is analysed by CD107a staining of resting NK cells (Figure 4B) to reveal the other primary HLH variants (FHL3-5).

Using a cut-off value of 5% this assay showed a sensitivity of 96% and a specificity of 88% for the detection of an inherited degranulation defect (51).

Additional flow cytometric studies have also been proven useful in the diagnostic work-up of HLH patients.

In cases of normal XIAP protein expression but high clinical suspicion for XLP2 the function of XIAP can be tested by stimulation of monocytes with muramyl dipeptides (L18-MDP) and analysis of TNFa expression by flow cytometry (55).

CONCLUSIONS

Flow cytometry is a highly valuable and versatile applicable diagnostic tool in the diagnostics and study of primary immunodeficiencies.

It has been contributing vitally to our understanding of the pathophysiology of these disorders and these findings have been translated into clinical diagnostic testing at a fast pace.

Given the still growing diversity of known PIDs on the one hand and the rarity of each of these disorders on the other, flow cytometry still proves to be one method of choice as it can be easily adapted to detect novel cellular pathologies in immune cells with manageable effort and costs.

Nevertheless, the diagnostic delay is still a major clinical problem in PIDs that needs to be addressed

Figure 4A **Analysis of intracellular XIAP expression in a patient with suspected X-linked lymphoproliferative syndrome showing severely reduced XIAP expression in NK and T-cells of the patient as compared to control (middle and right panels)**

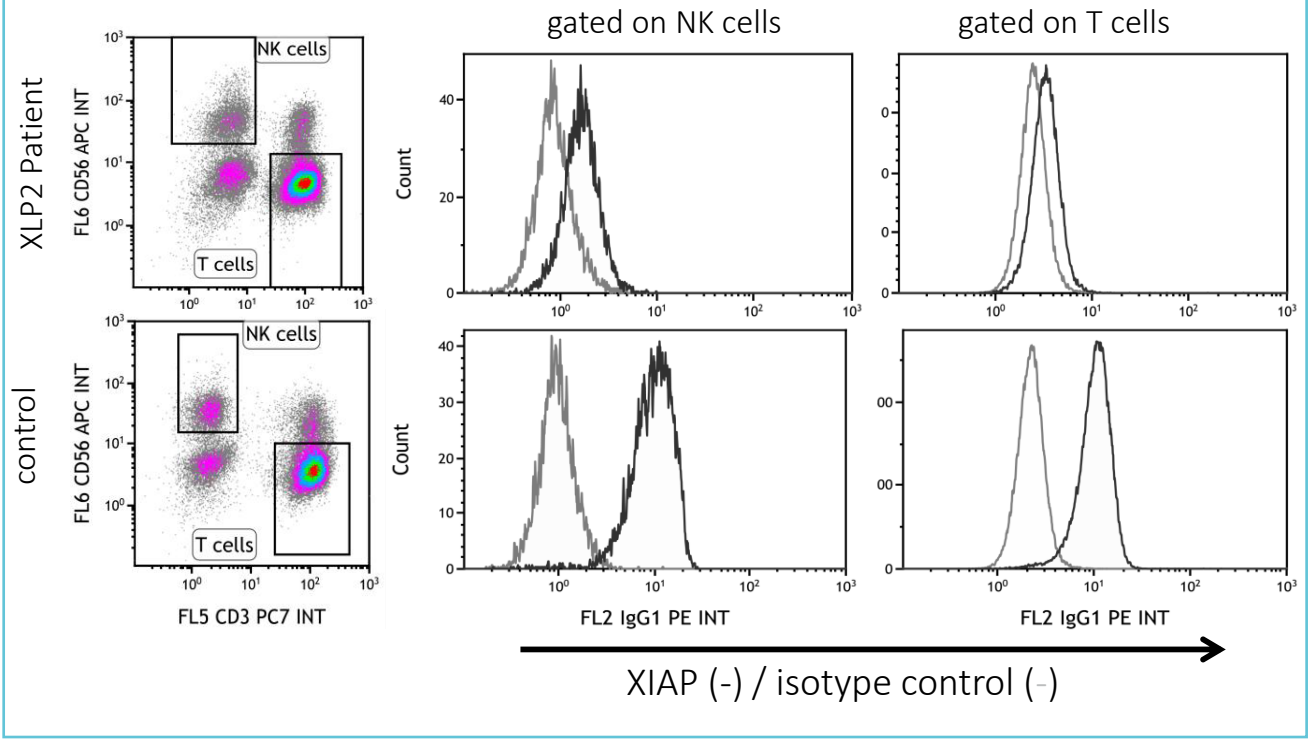
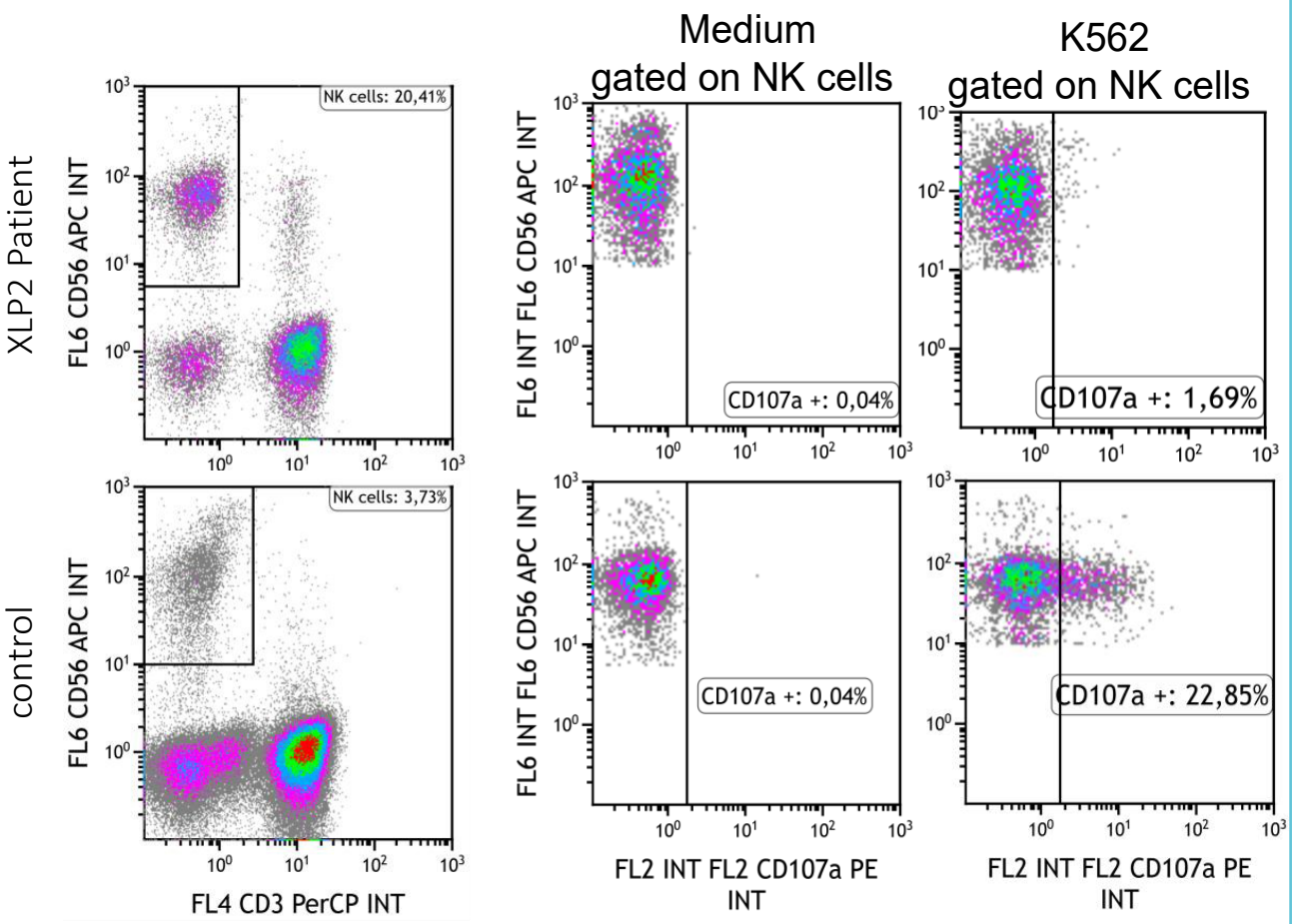


Figure 4B **Analysis of spontaneous degranulation of resting NK cells upon exposure to K562 target cells by staining for the cytotoxic granule associated protein CD107a in a patient with suspected FHL and a control showing impaired CD107a surface expression in the patient, indicating a defect in degranulation (right upper panel)**



by raising awareness and improvement of the flow cytometric diagnostic machinery.

❖❖❖❖❖

Compliance with ethical standards

Informed consent was obtained from all patients or their legal guardians and the study was done in compliance with the ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki.

Authors' disclosures

All authors declare they have no conflict of interest related to this manuscript.

Acknowledgements

We thank the patients and their families and we are grateful to the teams of the CCI Advanced Diagnostic Unit and the Diagnostic Laboratory of Rheumatology and Clinical Immunology for their excellent work.

REFERENCES

1. Picard C, Bobby Gaspar H, Al-Herz W, Bousfiha A, Casanova J-L, Chatila T, et al. International Union of Immunological Societies: 2017 Primary Immunodeficiency Diseases Committee Report on Inborn Errors of Immunity. J Clin Immunol. 2018;38(1):96–128.

2. Bousfiha A, Jeddane L, Picard C, Ailal F, Bobby Gaspar H, Al-Herz W, et al. The 2017 IUIS Phenotypic Classification for Primary Immunodeficiencies. J Clin Immunol. 2018;38(1):129–43.

3. Bruton OC. Agammaglobulinemia. Pediatrics. 1952; 9 (6):722–8.

4. Winkelstein JA, Marino MC, Lederman HM, Jones SM, Sullivan K, Burks AW, et al. X-linked agammaglobulinemia: report on a United States registry of 201 patients. Medicine (Baltimore). 2006;85(4):193–202.

5. Vetrie D, Vorechovsky I, Sideras P, Holland J, Davies A, Flinter F, et al. The gene involved in X-linked agammaglobulinaemia is a member of the src family of protein-tyrosine kinases. Nature. 1993;361(6409):226–33.

6. Tsukada S, Saffran DC, Rawlings DJ, Parolini O, Allen RC, Klisak I, et al. Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. Cell. 1993;72(2):279–90.

7. Noordzij JG, de Bruin-Versteeg S, Comans-Bitter WM, Hartwig NG, Hendriks RW, de Groot R, et al. Composition of precursor B-cell compartment in bone marrow from patients with X-linked agammaglobulinemia compared with healthy children. Pediatr Res. 2002;51(2):159–68.

8. Futatani T, Miyawaki T, Tsukada S, Hashimoto S, Kunikata T, Arai S, et al. Deficient expression of Bruton's tyrosine kinase in monocytes from X-linked agammaglobulinemia as evaluated by a flow cytometric analysis and its clinical application to carrier detection. Blood. 1998;91(2): 595–602.

9. Kanegane H, Futatani T, Wang Y, Nomura K, Shinozaki K, Matsukura H, et al. Clinical and mutational characteristics of X-linked agammaglobulinemia and its carrier identified by flow cytometric assessment combined with genetic analysis. J Allergy Clin Immunol. 2001;108(6):1012–20.

10. Rich R (ed.). Core Laboratory Technologies in Clinical Immunology - 1st Edition. https://www.elsevier.com/books/core-laboratory-technologies-in-clinical-immunology/rich/978-0-323-66149-2

11. Berglöf A, Turunen JJ, Gissberg O, Bestas B, Blomberg KEM, Smith CIE. Agammaglobulinemia: causative mutations and their implications for novel therapies. Expert Rev Clin Immunol. 2013;9(12):1205–21.

12. Conley ME, Notarangelo LD, Etzioni A. Diagnostic criteria for primary immunodeficiencies. Representing PAGID (Pan-American Group for Immunodeficiency) and ESID (European Society for Immunodeficiencies). Clin Immunol Orlando Fla. 1999;93(3):190–7.

13. Ameratunga R, Brewerton M, Slade C, Jordan A, Gillis D, Steele R, et al. Comparison of diagnostic criteria for common variable immunodeficiency disorder. Front Immunol. 2014;5:415.

14. Ameratunga R, Woon S-T, Gillis D, Koopmans W, Steele R. New diagnostic criteria for common variable immune deficiency (CVID), which may assist with decisions to treat with intravenous or subcutaneous immunoglobulin. Clin Exp Immunol. 2013;174(2):203–11.

15. Bonilla FA, Barlan I, Chapel H, Costa-Carvalho BT, Cunningham-Rundles C, de la Morena MT, et al. International Consensus Document (ICON): Common Variable Immunodeficiency Disorders. J Allergy Clin Immunol Pract. 2016;4(1):38–59.

16. Brouet JC, Chedeville A, Fermand JP, Royer B. Study of the B cell memory compartment in common variable immunodeficiency. Eur J Immunol. 2000;30(9):2516–20.

17. Warnatz K, Denz A, Dräger R, Braun M, Groth C, Wolff-Vorbeck G, et al. Severe deficiency of switched memory B cells (CD27(+)IgM(-)IgD(-)) in subgroups of patients with common variable immunodeficiency: a new approach to classify a heterogeneous disease. Blood. 2002;99(5): 1544–51.

18. Agematsu K, Futatani T, Hokibara S, Kobayashi N, Takamoto M, Tsukada S, et al. Absence of memory B cells in patients with common variable immunodeficiency. Clin Immunol Orlando Fla. 2002;103(1):34–42.

19. Rakhmanov M, Keller B, Gutenberger S, Foerster C, Hoenig M, Driessen G, et al. Circulating CD21low B cells in common variable immunodeficiency resemble tissue homing, innate-like B cells. Proc Natl Acad Sci U S A. 2009;106(32): 13451–6.

20. Piqueras B, Lavenu-Bombled C, Galicier L, Bergeron-van der Cruyssen F, Mouthon L, Chevret S, et al. Common variable immunodeficiency patient classification based on impaired B cell memory differentiation correlates with clinical aspects. J Clin Immunol. 2003;23(5): 385–400.

21. Wehr C, Kivioja T, Schmitt C, Ferry B, Witte T, Eren E, et al. The EUROclass trial: defining subgroups in common variable immunodeficiency. Blood. 2008;111(1):77–85.

22. Resnick ES, Moshier EL, Godbold JH, Cunningham-Rundles C. Morbidity and mortality in common variable immune deficiency over 4 decades. Blood. 2012;119(7): 1650–7.

23. Wehr C, Eibel H, Masilamani M, Illges H, Schlesier M, Peter H-H, et al. A new CD21low B cell population in the

peripheral blood of patients with SLE. Clin Immunol Orlando Fla. 2004;113(2):161–71.

24. Terrier B, Joly F, Vazquez T, Benech P, Rosenzwajg M, Carpentier W, et al. Expansion of functionally anergic CD21-/low marginal zone-like B cell clones in hepatitis C virus infection-related autoimmunity. J Immunol Baltim Md 1950. 2011;187(12):6550–63.

25. Saadoun D, Terrier B, Bannock J, Vazquez T, Massad C, Kang I, et al. Expansion of autoreactive unresponsive CD21-/low B cells in Sjögren's syndrome-associated lymphoproliferation. Arthritis Rheum. 2013;65(4):1085–96.

26. Malphettes M, Gérard L, Carmagnat M, Mouillot G, Vince N, Boutboul D, et al. Late-onset combined immune deficiency: a subset of common variable immunodeficiency with severe T cell defect. Clin Infect Dis Off Publ Infect Dis Soc Am. 2009;49(9):1329–38.

27. Giovannetti A, Pierdominici M, Mazzetta F, Marziali M, Renzi C, Mileo AM, et al. Unravelling the complexity of T cell abnormalities in common variable immunodeficiency. J Immunol Baltim Md 1950. 2007;178(6):3932–43.

28. Grimbacher B, Hutloff A, Schlesier M, Glocker E, Warnatz K, Dräger R, et al. Homozygous loss of ICOS is associated with adult-onset common variable immunodeficiency. Nat Immunol. 2003;4(3):261–8.

29. van Zelm MC, Reisli I, van der Burg M, Castaño D, van Noesel CJM, van Tol MJD, et al. An antibody-deficiency syndrome due to mutations in the CD19 gene. N Engl J Med. 2006;354(18):1901–12.

30. Warnatz K, Salzer U, Rizzi M, Fischer B, Gutenberger S, Böhm J, et al. B-cell activating factor receptor deficiency is associated with an adult-onset antibody deficiency syndrome in humans. Proc Natl Acad Sci U S A. 2009;106(33):13945–50.

31. Vinh DC, Patel SY, Uzel G, Anderson VL, Freeman AF, Olivier KN, et al. Autosomal dominant and sporadic monocytopenia with susceptibility to mycobacteria, fungi, papillomaviruses, and myelodysplasia. Blood. 2010;115(8):1519–29.

32. Bigley V, Haniffa M, Doulatov S, Wang X-N, Dickinson R, McGovern N, et al. The human syndrome of dendritic cell, monocyte, B and NK lymphoid deficiency. J Exp Med. 2011;208(2):227–34.

33. Dickinson RE, Griffin H, Bigley V, Reynard LN, Hussain R, Haniffa M, et al. Exome sequencing identifies GATA-2 mutation as the cause of dendritic cell, monocyte, B and NK lymphoid deficiency. Blood. 2011;118(10):2656–8.

34. Hsu AP, Sampaio EP, Khan J, Calvo KR, Lemieux JE, Patel SY, et al. Mutations in GATA2 are associated with the autosomal dominant and sporadic monocytopenia and mycobacterial infection (MonoMAC) syndrome. Blood. 2011;118(10):2653–5.

35. Ostergaard P, Simpson MA, Connell FC, Steward CG, Brice G, Woollard WJ, et al. Mutations in GATA2 cause primary lymphedema associated with a predisposition to acute myeloid leukemia (Emberger syndrome). Nat Genet. 2011;43(10):929–31.

36. Hahn CN, Chong C-E, Carmichael CL, Wilkins EJ, Brautigan PJ, Li X-C, et al. Heritable GATA2 mutations associated with familial myelodysplastic syndrome and acute myeloid leukemia. Nat Genet. 2011;43(10):1012–7.

37. Cortés-Lavaud X, Landecho MF, Maicas M, Urquiza L, Merino J, Moreno-Miralles I, et al. GATA2 germline mutations impair GATA2 transcription, causing haploinsufficiency: functional analysis of the p.Arg396Gln mutation. J Immunol Baltim Md 1950. 2015;194(5):2190–8.

38. Kazenwadel J, Secker GA, Liu YJ, Rosenfeld JA, Wildin RS, Cuellar-Rodriguez J, et al. Loss-of-function germline GATA2 mutations in patients with MDS/AML or MonoMAC syndrome and primary lymphedema reveal a key role for GATA2 in the lymphatic vasculature. Blood. 2012;119(5):1283–91.

39. Kazenwadel J, Betterman KL, Chong C-E, Stokes PH, Lee YK, Secker GA, et al. GATA2 is required for lymphatic vessel valve development and maintenance. J Clin Invest. 2015;125(8):2979–94.

40. Dickinson RE, Milne P, Jardine L, Zandi S, Swierczek SI, McGovern N, et al. The evolution of cellular deficiency in GATA2 mutation. Blood. 2014;123(6):863–74.

41. Spinner MA, Sanchez LA, Hsu AP, Shaw PA, Zerbe CS, Calvo KR, et al. GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. Blood. 2014;123(6):809–21.

42. Sepulveda FE, de Saint Basile G. Hemophagocytic syndrome: primary forms and predisposing conditions. Curr Opin Immunol. 2017;49:20–6.

43. Stepp SE, Dufourcq-Lagelouse R, Le Deist F, Bhawan S, Certain S, Mathew PA, et al. Perforin gene defects in familial hemophagocytic lymphohistiocytosis. Science. 1999;286(5446):1957–9.

44. Feldmann J, Callebaut I, Raposo G, Certain S, Bacq D, Dumont C, et al. Munc13-4 is essential for cytolytic granules fusion and is mutated in a form of familial hemophagocytic lymphohistiocytosis (FHL3). Cell. 2003;115(4):461–73.

45. zur Stadt U, Schmidt S, Kasper B, Beutel K, Diler AS, Henter J-I, et al. Linkage of familial hemophagocytic lymphohistiocytosis (FHL) type-4 to chromosome 6q24 and identification of mutations in syntaxin 11. Hum Mol Genet. 2005;14(6):827–34.

46. zur Stadt U, Rohr J, Seifert W, Koch F, Grieve S, Pagel J, et al. Familial hemophagocytic lymphohistiocytosis type 5 (FHL-5) is caused by mutations in Munc18-2 and impaired

binding to syntaxin 11. Am J Hum Genet. 2009;85(4): 482–92.

47. Ménasché G, Pastural E, Feldmann J, Certain S, Ersoy F, Dupuis S, et al. Mutations in RAB27A cause Griscelli syndrome associated with haemophagocytic syndrome. Nat Genet. 2000;25(2):173–6.

48. Nagle DL, Karim MA, Woolf EA, Holmgren L, Bork P, Misumi DJ, et al. Identification and mutation analysis of the complete gene for Chediak-Higashi syndrome. Nat Genet. 1996;14(3):307–11.

49. Coffey AJ, Brooksbank RA, Brandau O, Oohashi T, Howell GR, Bye JM, et al. Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. Nat Genet. 1998;20(2):129–35.

50. Nichols KE, Harkin DP, Levitz S, Krainer M, Kolquist KA, Genovese C, et al. Inactivating mutations in an SH2 domain-encoding gene in X-linked lymphoproliferative syndrome. Proc Natl Acad Sci U S A. 1998;95(23):13765–70.

51. Bryceson YT, Pende D, Maul-Pavicic A, Gilmour KC, Ufheil H, Vraetz T, et al. A prospective evaluation of degranulation assays in the rapid diagnosis of familial hemophagocytic syndromes. Blood. 2012;119(12): 2754–63.

52. Ammann S, Lehmberg K, Zur Stadt U, Klemann C, Bode SFN, Speckmann C, et al. Effective Immunological Guidance of Genetic Analyses Including Exome Sequencing in Patients Evaluated for Hemophagocytic Lymphohistiocytosis. J Clin Immunol. 2017;37(8):770–80.

53. Kogawa K, Lee SM, Villanueva J, Marmer D, Sumegi J, Filipovich AH. Perforin expression in cytotoxic lymphocytes from patients with hemophagocytic lymphohistiocytosis and their family members. Blood. 2002;99(1):61–6.

54. Gifford CE, Weingartner E, Villanueva J, Johnson J, Zhang K, Filipovich AH, et al. Clinical flow cytometric screening of SAP and XIAP expression accurately identifies patients with SH2D1A and XIAP/BIRC4 mutations. Cytometry B Clin Cytom. 2014;86(4):263–71.

55. Ammann S, Elling R, Gyrd-Hansen M, Dückers G, Bredius R, Burns SO, et al. A new functional assay for the diagnosis of X-linked inhibitor of apoptosis (XIAP) deficiency. Clin Exp Immunol. 2014;176(3):394–400.