



Application of standardized flowcytometry as a first step in the diagnostics of PID

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Department | central diagnostic laboratory, MUMC+

Standardized flow cytometry in the diagnostics of primary immune deficiencies

- The value of flowcytometry in the current diagnostic process
 - Severe combined immune deficiency (SCID)
 - Common variable immune deficiency (CVID)
- Future applications of flowcytometry in immune deficiencies
 - Immune monitoring as a tool to predict complications
 - Proof of concept: Fingerprint of viral infection in a healthy control, GVHD, CMV
 - Complications in CVID → profylaxis?
 - Immune monitoring as a tool for adjusted therapy in secondary immune deficiencies



Protocol for diagnosing primary immunodeficiency

Patient-centred screening for primary immunodeficiency, a multi-stage diagnostic protocol designed for non-immunologists: 2011 update
Clin Exp Immunol 2012; **167**(1):108-19.

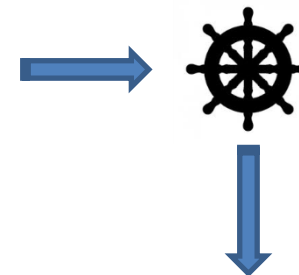


The clinical presentations of PID

- Recurrent ENT and airway infections
- Failure to thrive from early infancy
- Recurrent pyogenic infections
- Unusual infections or unusually severe course of infections
- Recurrent infections with the same type of pathogen
- Autoimmune or chronic inflammatory disease; lymphoproliferation
- Characteristic combinations of clinical features (eponymous syndromes)
- Angioedema



1 component of immune system



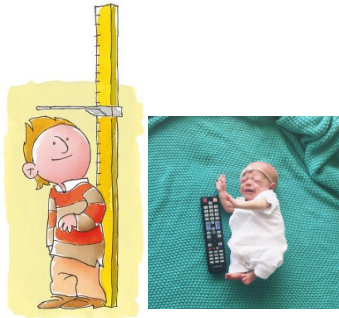
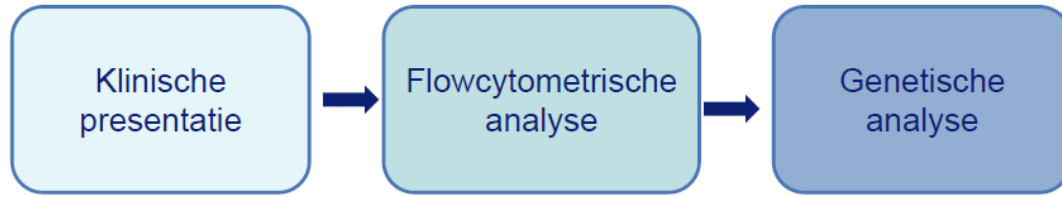
Typical infections
Typical symptoms

Protocol for diagnosing primary immunodeficiency

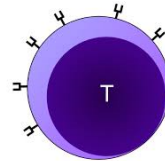
Patient-centred screening for primary immunodeficiency, a multi-stage diagnostic protocol designed for non-immunologists: 2013 update
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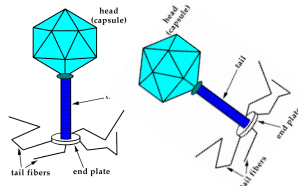
Severe combined immune deficiency (SCID)



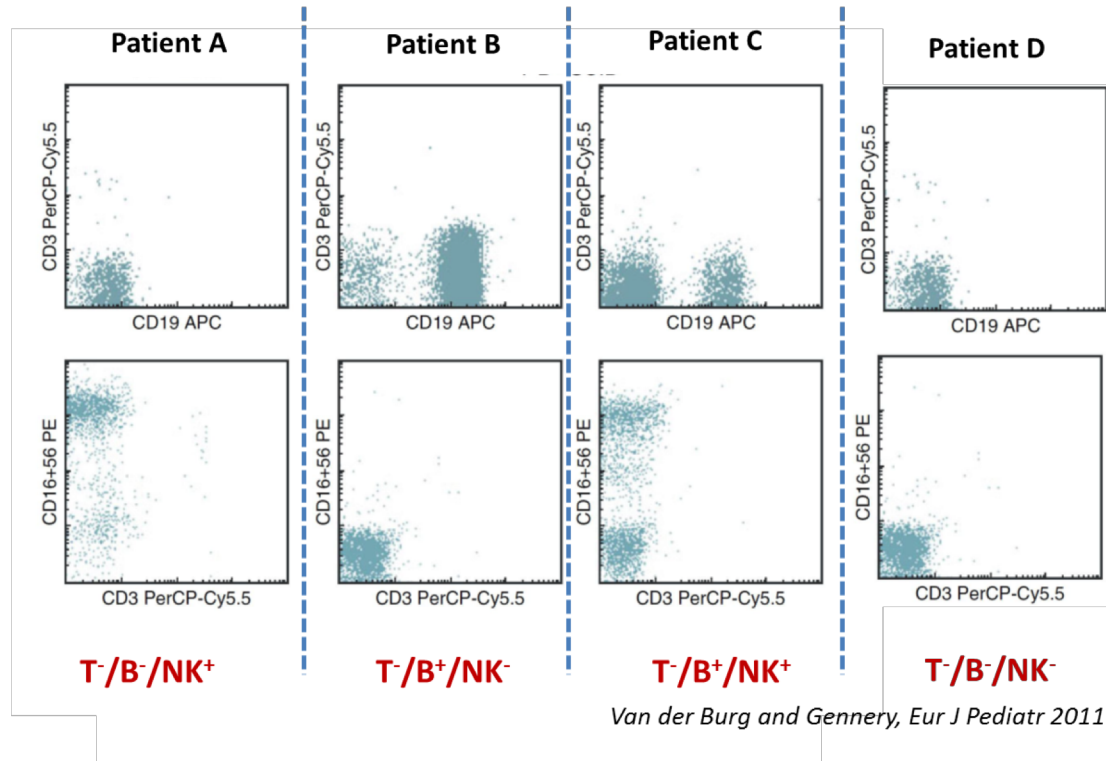
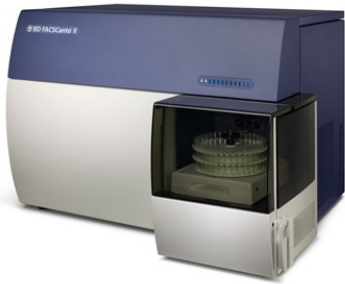
groeiachterstand



T cel



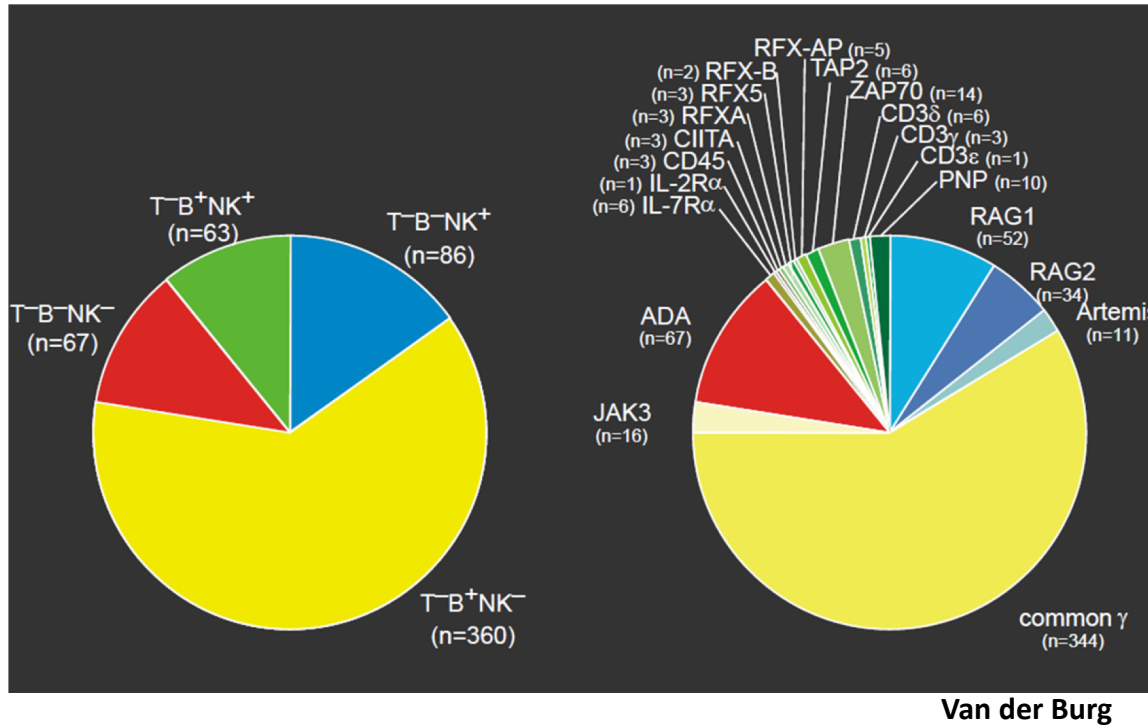
CMV, EBV, VSV



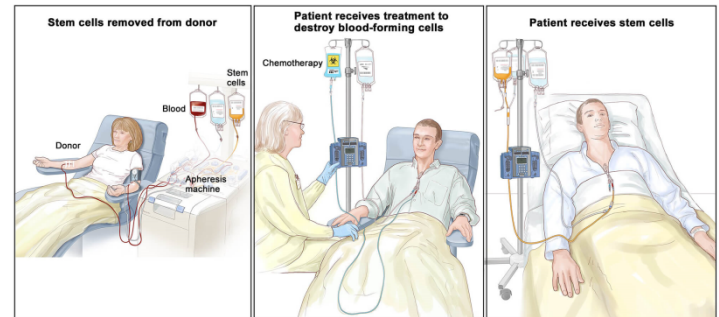
Importance of genetic conformation in SCID

- Genetic counseling and analysis of carriers in the family
- Early detection of affected siblings
- Patient compliance

Flow cytometric phenotype → link to the genetic defect



TIME IS OF THE ESSENCE



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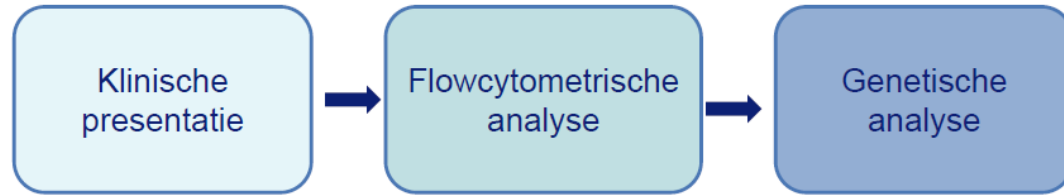
Flow cytometry → Swift diagnosis
Perinatal screening → TREC-analysis

Protocol for diagnosing primary immunodeficiency

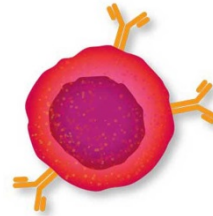
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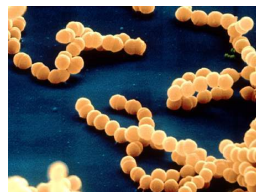
Common variable immune deficiency (CVID)



**KNO infecties
Bovenste luchtwegen**

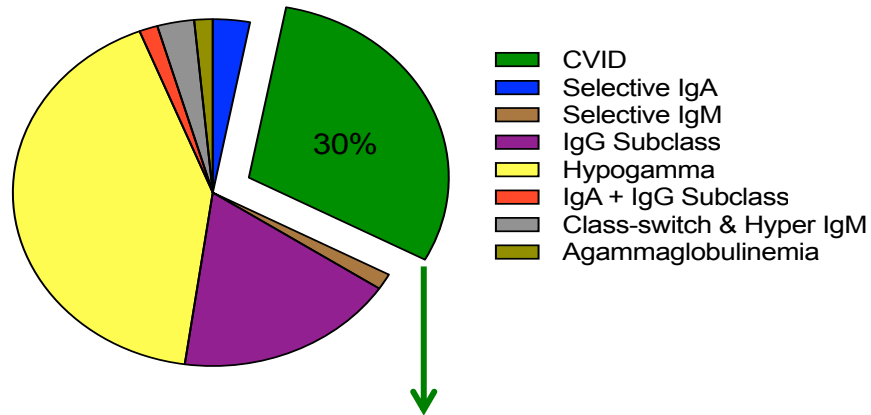


B cell

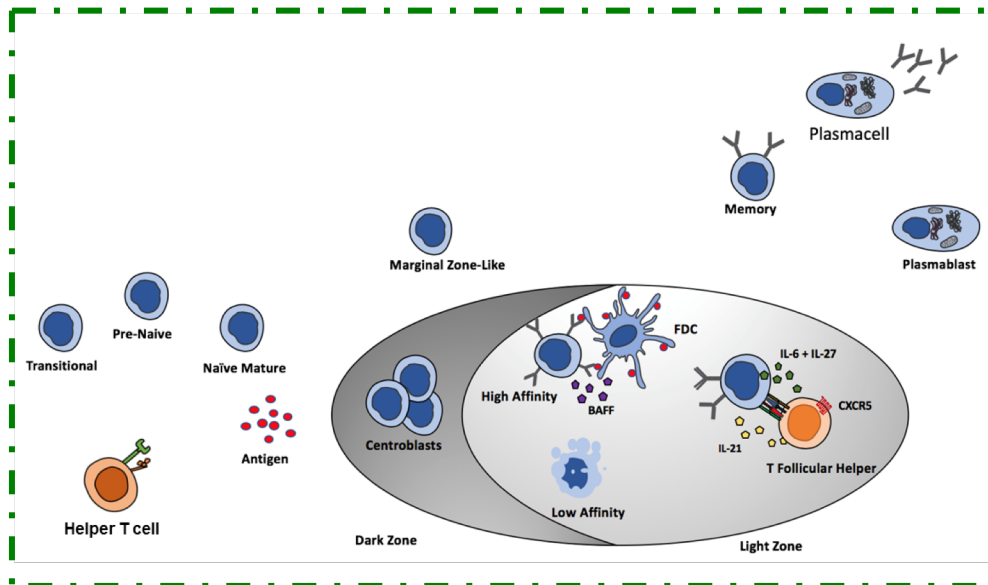


**Bacteriën
Streptococci
Haemophilus infl**

Common Variable Immunodeficiency

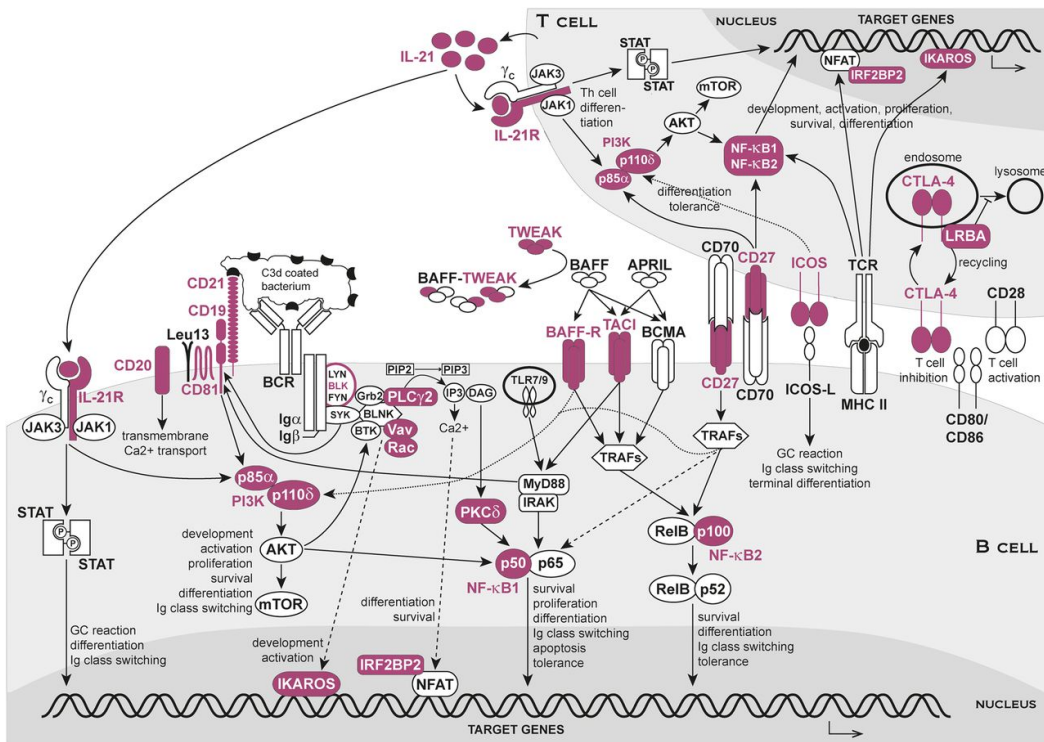
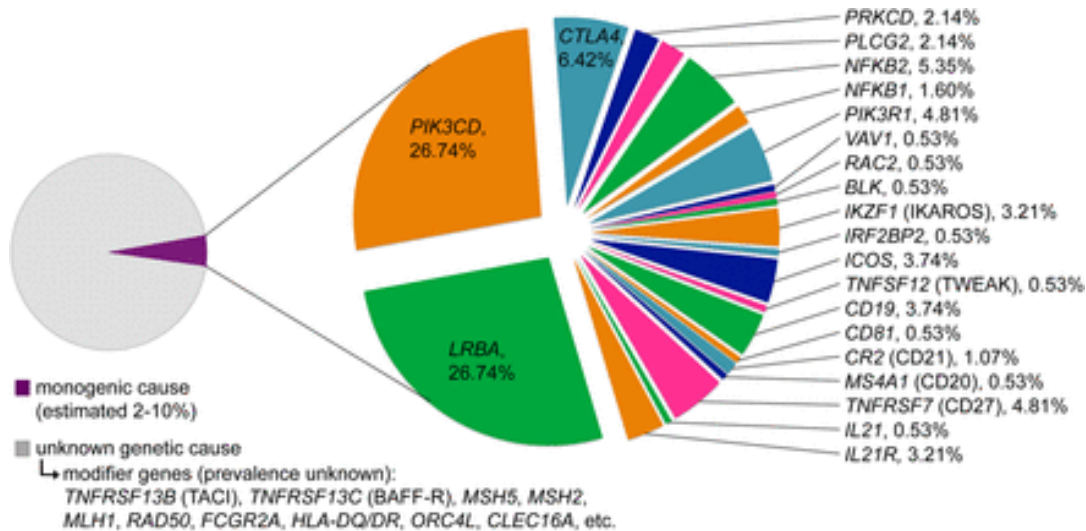


Defects in B cell maturation



- Primary antibody deficiency
- Insufficient production of IgG & IgA
- Impaired humoral responses
- Recurrent upper airway infections
- Underlying genetic defect ($\pm 20\%$)
- **Heterogeneity**

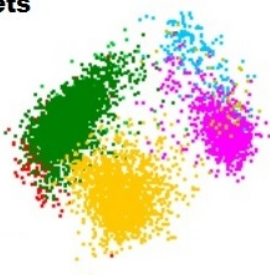
Genetic defect



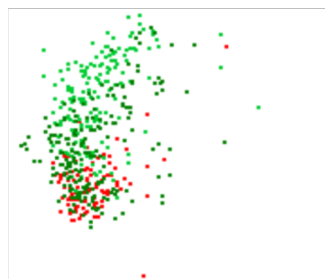
- Multiple genetic defects can cause CVID
- Not in all patients genetic defect is identified (20-30%)
- CVID causing genetic defects in both T cells and B cells

B Lymphocyte Subsets

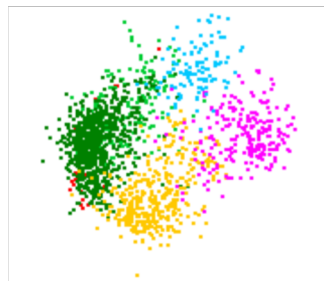
- Transitional
- Naive CD21-
- Naive CD21+
- Unswitched Memory
- Memory CD27+
- Memory CD27-
- Plasmablasts



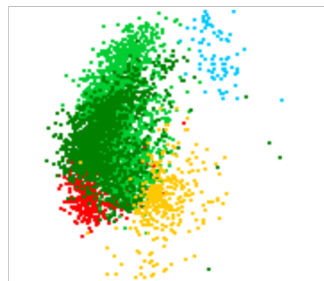
APS B Lymphocytes



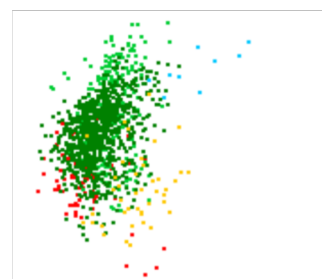
CVID I



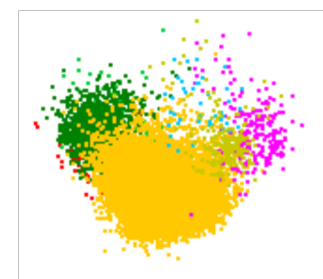
CVID II



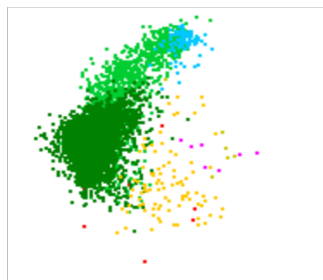
CVID III



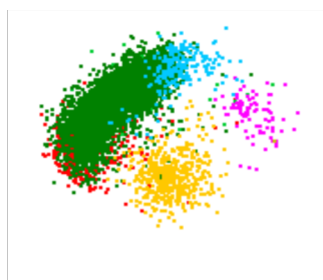
CVID IV



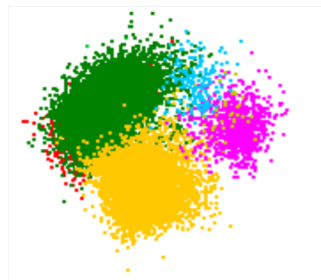
CVID V



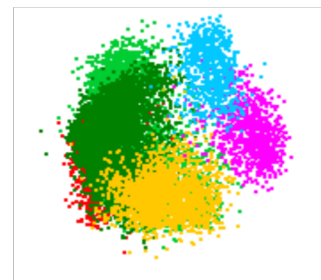
CVID VI



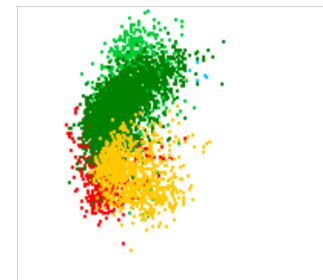
CVID VII



CVID VIII



CVID IX



CVID X



EuroFlow



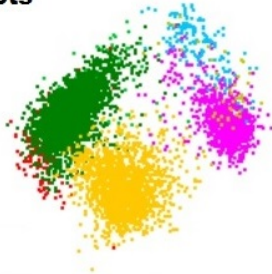
Flow Cytometry Data Analysis Software

Presented by Roberto Juanes Juanes

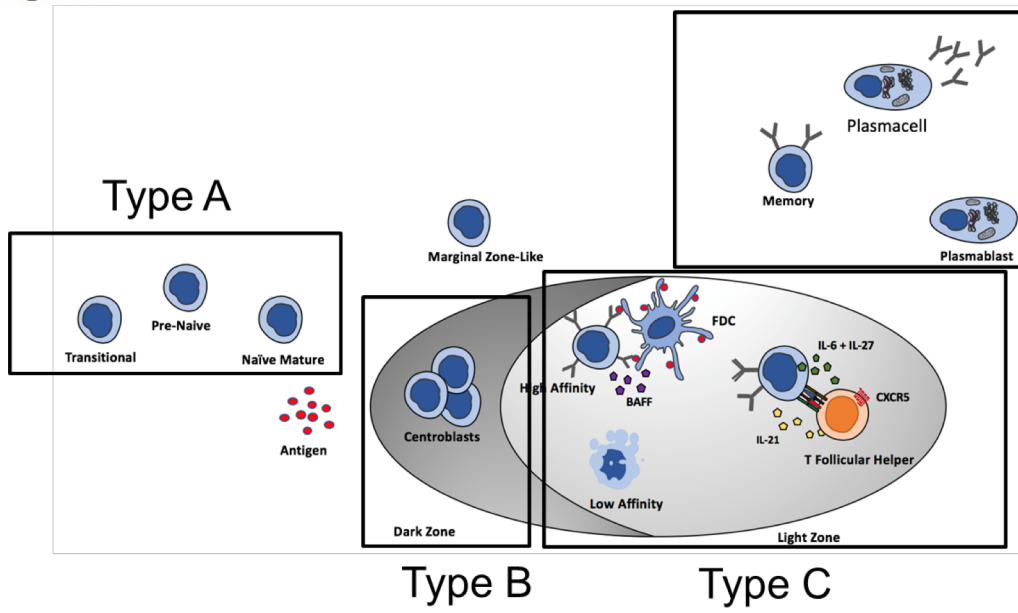
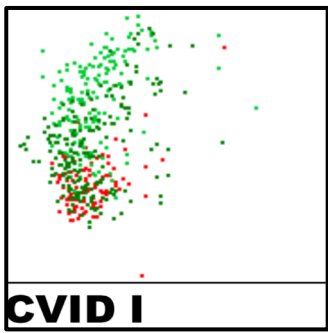
Infinicyt™ software available in the US from ALPCO through a partnership with cytogenos

B Lymphocyte Subsets

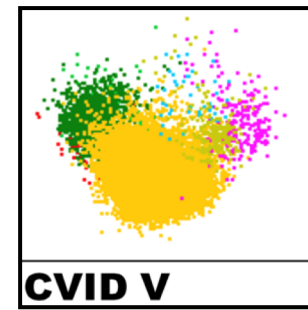
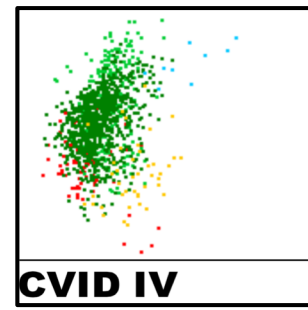
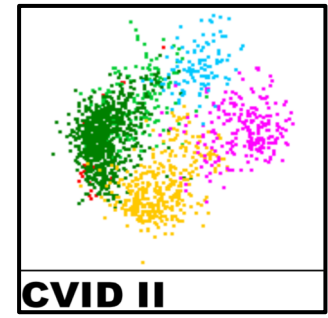
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APS B Lymphocytes



Type D

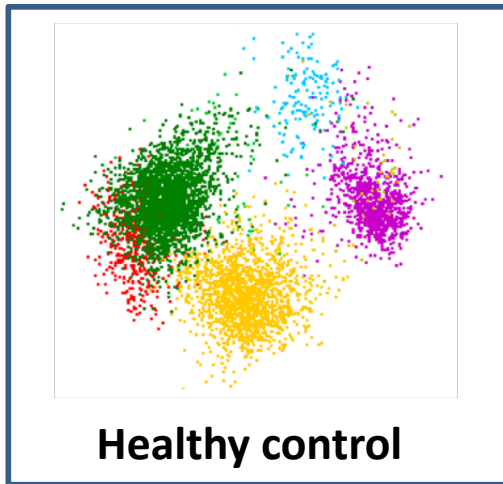


CVID IV

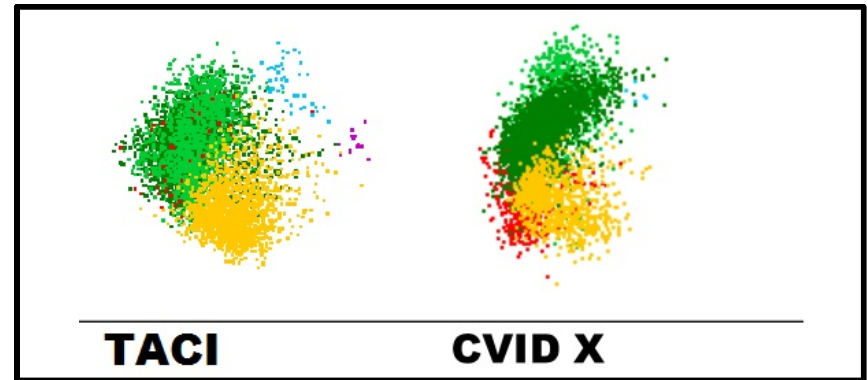
CVID V

Patient with known genetic defect

B cells



B cells



- Identification of patients based on the immune phenotype with genetic defects in the same phase of B cell development?
- Similar clinical phenotype?

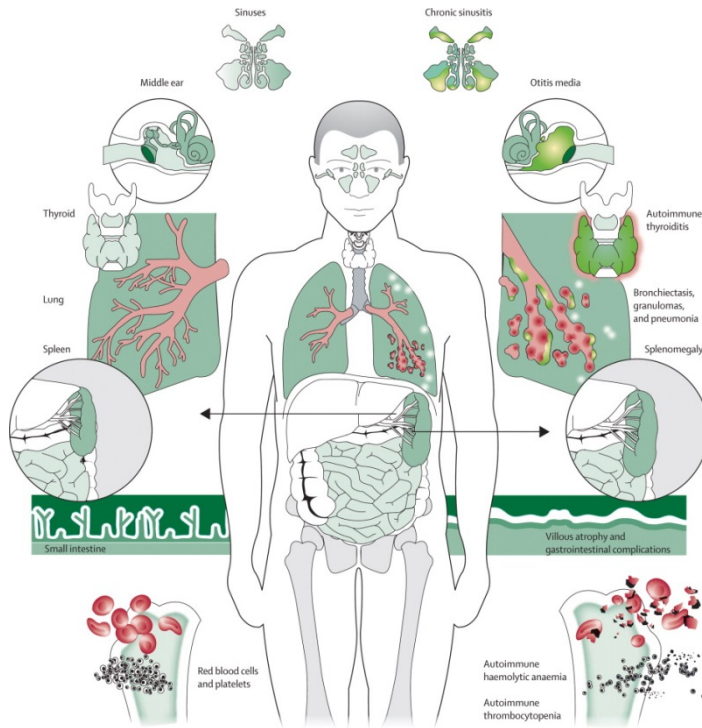
	TACI (1950)	CVID X (1971)
IgG	< 1,1 g/L	< 1,1 g/L
IgA	< 0,5 g/L	< 0,1 g/L
IgM	< 0,1 g/L	0,17 g/L
Infections	Sinopulmonary	Sinopulmonary Gastro-intestinal
Auto immune disease	-	-
Granulomatous infiltration	-	-
Lymphadenopathy/ Splenomegaly	Splenomegaly	Generalized + splenomegaly
Malignancies	Hodgkin	- (WES nl)

Importance of flow in the current diagnostic process

Standardized flow cytometry (euroflow)

- Allows localization of defects in the immune system in CVID
 - Based on immune phenotype → localisation of the genetic defect
 - Also applicable in case the genetic defect cannot be identified (for CVID <75% of cases?)
- **Patient database** allows comparison with other patients (most PID are rare diseases)
- **In CVID knowing the defect does not (yet) have a treatment consequence**

Importance of flow in the future diagnostic process



Park et al., Lancet (2008) 372,489-502

Complications in CVID:

1. Infection
2. Granuloma
3. Autoimmunity
4. Lymphocytic infiltration/proliferation
5. Malignancy



1. Associate with different immune responses!
2. Require therapeutic intervention

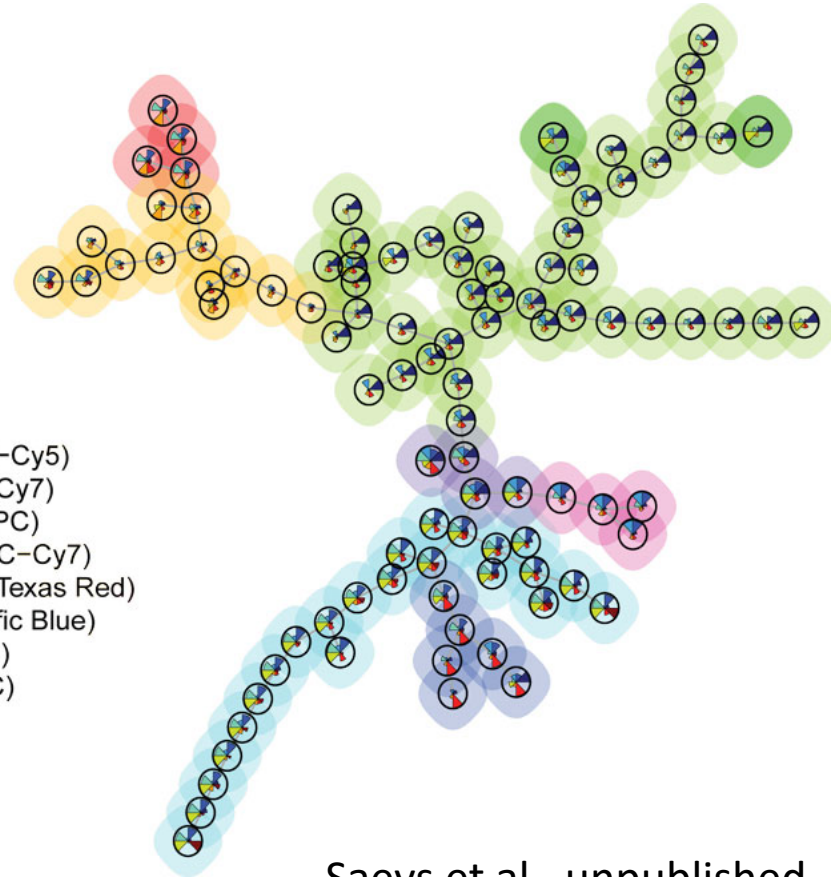
Is it possible to predict whether patient is at risk for complications by immune monitoring

Importance of flow in the future diagnostic process



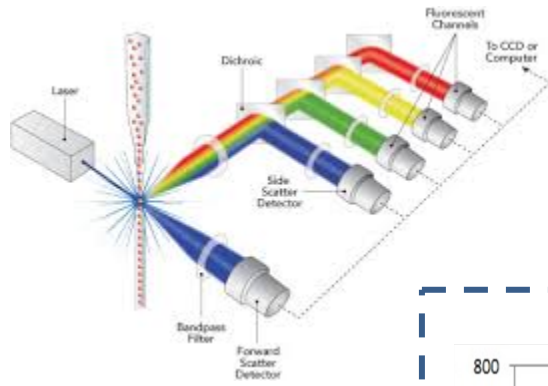
> 25 stainings

- CD19 (PE-Cy5)
- CD3 (PE-Cy7)
- TCRyd (APC)
- TCRb (APC-Cy7)
- CD4 (PE-Texas Red)
- CD8 (Pacific Blue)
- NK1/1 (PE)
- GFP (FITC)

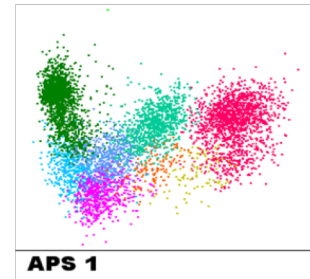
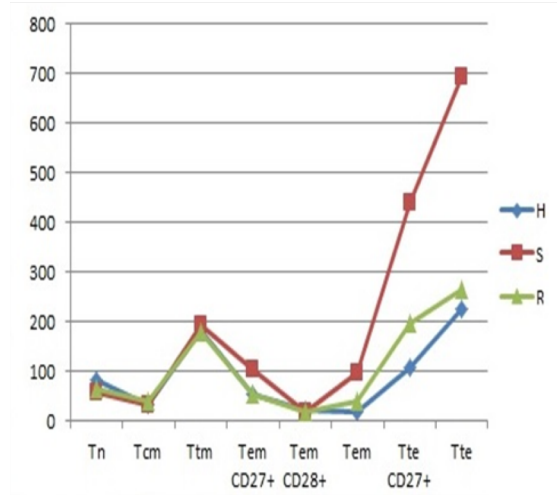


Saeys et al., unpublished

Proof of concept: visualization of immune responses with Euroflow



Viral infection: flu



APS 1



APS 1

Eyndhoven en Van Hulst, unpublished

GVHD or CMV after HSCT

Importance of flow in the future diagnostic process

- Flowcytometry not only for the diagnostic process but also for patient monitoring
 - predict complications
 - Tool for patient management

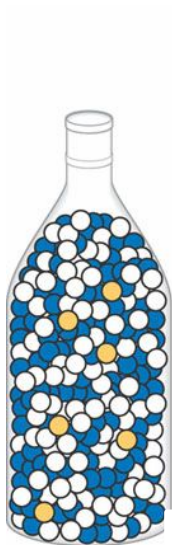
Type of clinical complication	Prevention	Screening	Treatment
Infectious	Ig replacement; prophylactic antibiotics; vaccination	Patients' awareness; sputum monitoring; routine visits	High dose Ig; therapeutic antibiotics
Pulmonary	Control of infection; high dose Ig	Spirometry; HRCT; routine visits	Endoscopic sinus surgery; inhaled corticosteroids; anti-inflammatory antibiotics; IL-2 therapy; B ₂ agonists; leukotriene receptor antagonists; lung transplantation
Lymphoproliferative	–	Lymph nodes biopsy; spirometry; imaging; routine visits	Systemic corticosteroids; hydroxychloroquine; immunosuppressive agents
Autoimmunity	Ig replacement?	CBC, diff PBS; thyroid examination and thyroid function; routine visits	Corticosteroids; anti-CD20 monoclonal antibodies; TNF- α inhibitors
Gastrointestinal	Control of infection, autoimmunity and lymphoproliferative complications	Upper and/or lower endoscopy and yearly ultrasonography; routine visits	Immunomodulators; TNF- α inhibitors
Neoplasia	<i>Helicobacter pylori</i> eradication; decreasing unnecessary irradiation	Routine cancer screening; screening by endoscopy; bone marrow examinations	Routine chemotherapy; rituximab protocols; surgical modalities; allogeneic stem cell transplantation

CBC: Complete blood count; HRCT: High-resolution computed tomography; Ig: Immunoglobulin; PBS: Peripheral blood smear.

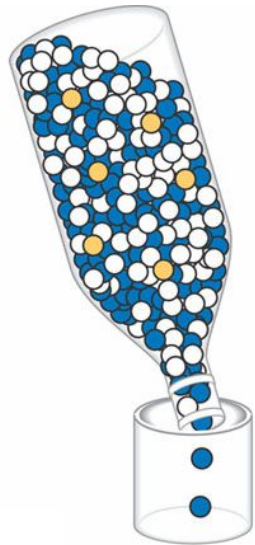
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- Autoreactive T cells
- CD4⁺ T cells
- CD8⁺ T cells

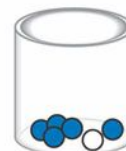
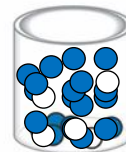
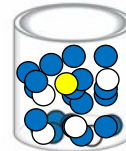
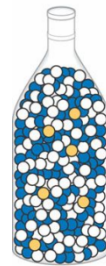


Original population

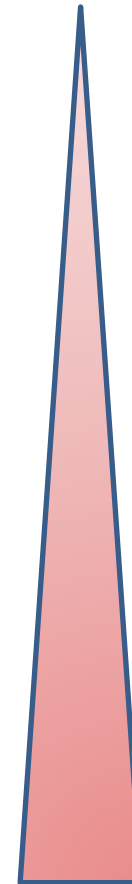


Bottlenecking event

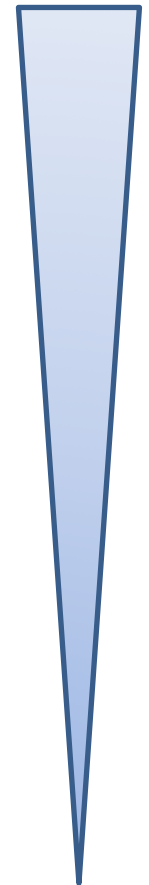
Immune suppression



Surviving population

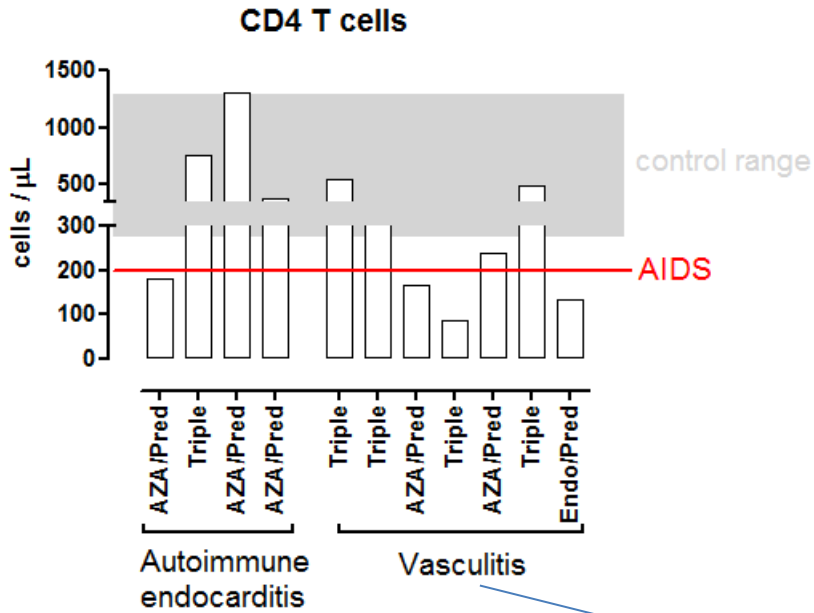


Immune compromised



Disease symptoms





Anca associated vasculitis

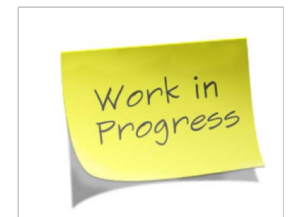
Azathioprine-Regimen

Pulsed-cyclophosphamide
Corticoids
Azathioprine maintenance

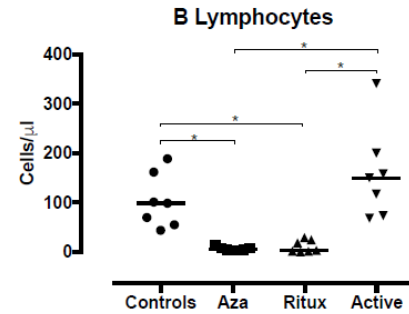
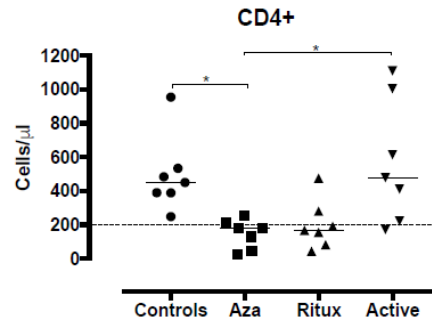
Rituximab-Regimen

Pulsed-cyclophosphamide
Corticoids
Rituximab

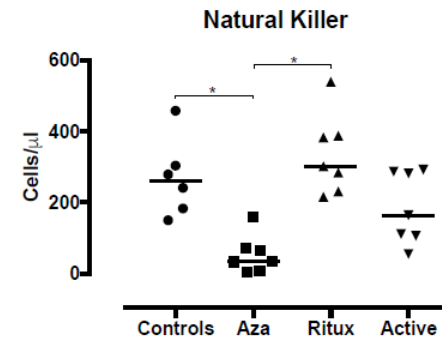
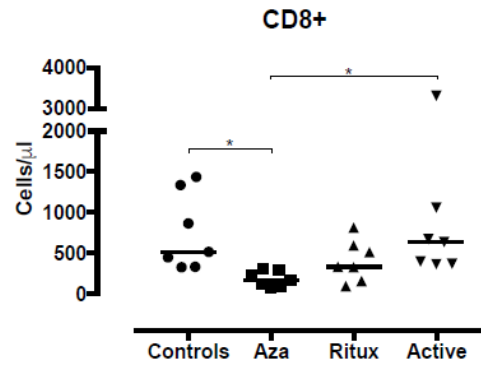
Immune profile
Proliferative responses
Cytokine secretion profile
TCR repertoire
TREC analysis



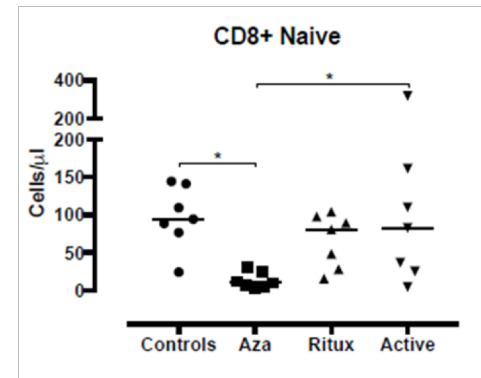
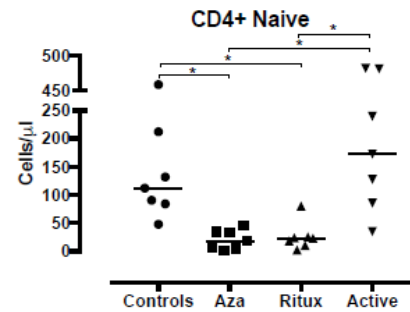
Disease promoting
immune populations



Other
immune populations



Future
Immune competence



Standardized immune phenotyping (Euroflow) can be a powerful tool for therapeutic immune monitoring

Immune monitoring after 2 standard immune suppressive regimens for AAV

- T (CD4 + CD8) and B cells are equally affected by both regimens
- Azathioprine → more severe impact on CD8+ and NK (viral defense + cancer)
- Naive T cells are not spared during immune suppression
- Therapy also associates with phenotypic alterations → functional?

Combining standardized immune phenotyping during therapy with

1. Patient outcome:

- Development of database of patients and controls
- Identify patients that are at risk for complications

Immune phenotyping will facilitate decision making during therapy AND will provide novel tools in the search of improved therapeutic regimens

Importance of flow in the diagnostic process

Standardized flow cytometry (euroflow) gives swift information on the status of the immune system

- Allows localization of defects in the immune system
 - Based on immune phenotype → **localisation of the genetic defect**
 - Also applicable in case the genetic defect cannot be identified (for CVID <75% of cases?)
 - Not always impact on treatment

Future application of flow cytometry → immune monitoring

- Predict clinical complications in primary immune deficiencies
 - impact on patient treatment (prophylaxis, antibiotics, steroids, ...)
- Secondary immune deficiencies
 - **Database:** Immune monitoring after a fixed time of standard immune suppression
 - + clinical follow-up of patient
 - Predict whether patient is at risk for complications

Central Diagnostic Laboratory:

NCJ de Wit

J Damoiseaux

J Vanderlocht

LC Van Eyndhoven

GRDT Van Hulst

A. Camman

Internal medicine

Infectious diseases:

SH Lowe

A. Oude Lashof

Clinical immunology

P. Van Paassen

J. Potjewijd

C. Nieuwhof

Euroflow consortium:

Prof. dr. JJM van Dongen

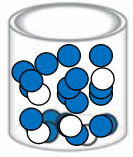
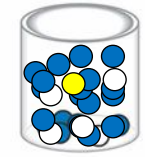
Prof. dr. M van der Burg



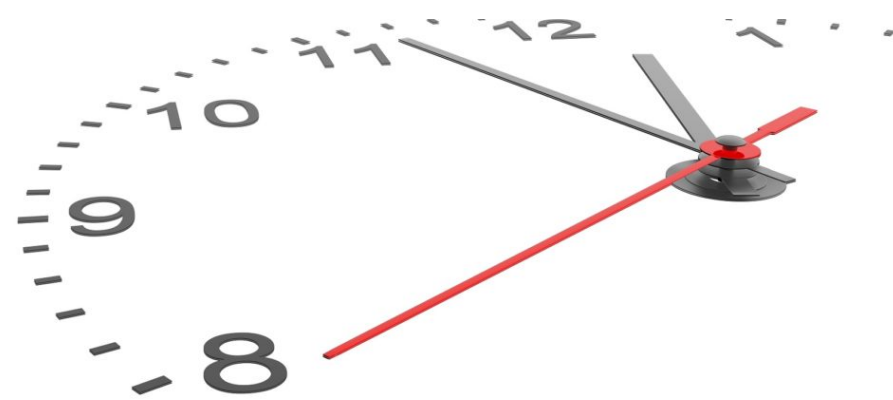
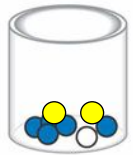
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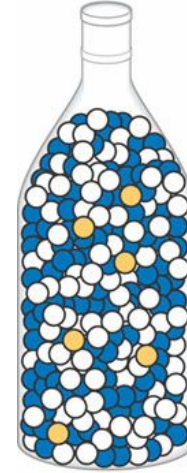


Maintenance therapy

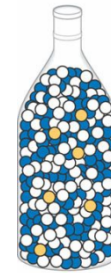


No therapy

Immune status



OR

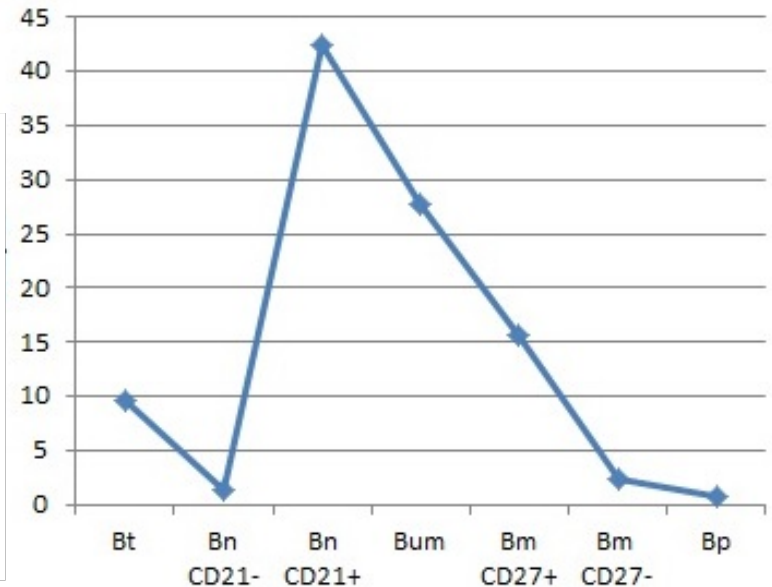
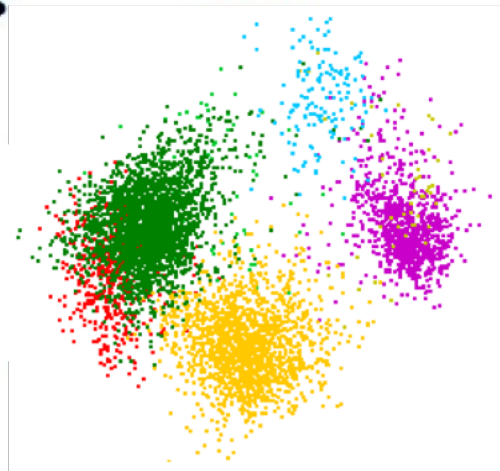


Quantitative or qualitative

Visualization of Data

B Lymphocyte Subsets

- Transitional
- Naive CD21-
- Naive CD21+
- Unswitched Memory
- Memory CD27+
- Memory CD27-
- Plasmablasts



APS B Lymphocytes

Automatic Population Separator

CSP B Lymphocytes

Connected Subsets Pattern

Clinical characteristics

	TACI (1950)	CVID X (1971)
IgG	< 1,1 g/L	< 1,1 g/L
IgA	< 0,5 g/L	< 0,1 g/L
IgM	< 0,1 g/L	0,17 g/L
Infections	Sinopulmonary	Sinopulmonary Gastro-intestinal
Auto immune disease	-	-
Granulomatous infiltration	-	-
Lymphadenopathy/ Splenomegaly	Splenomegaly	Generalized + splenomegaly
Malignancies	Hodgkin	- (WES nl)



WBC

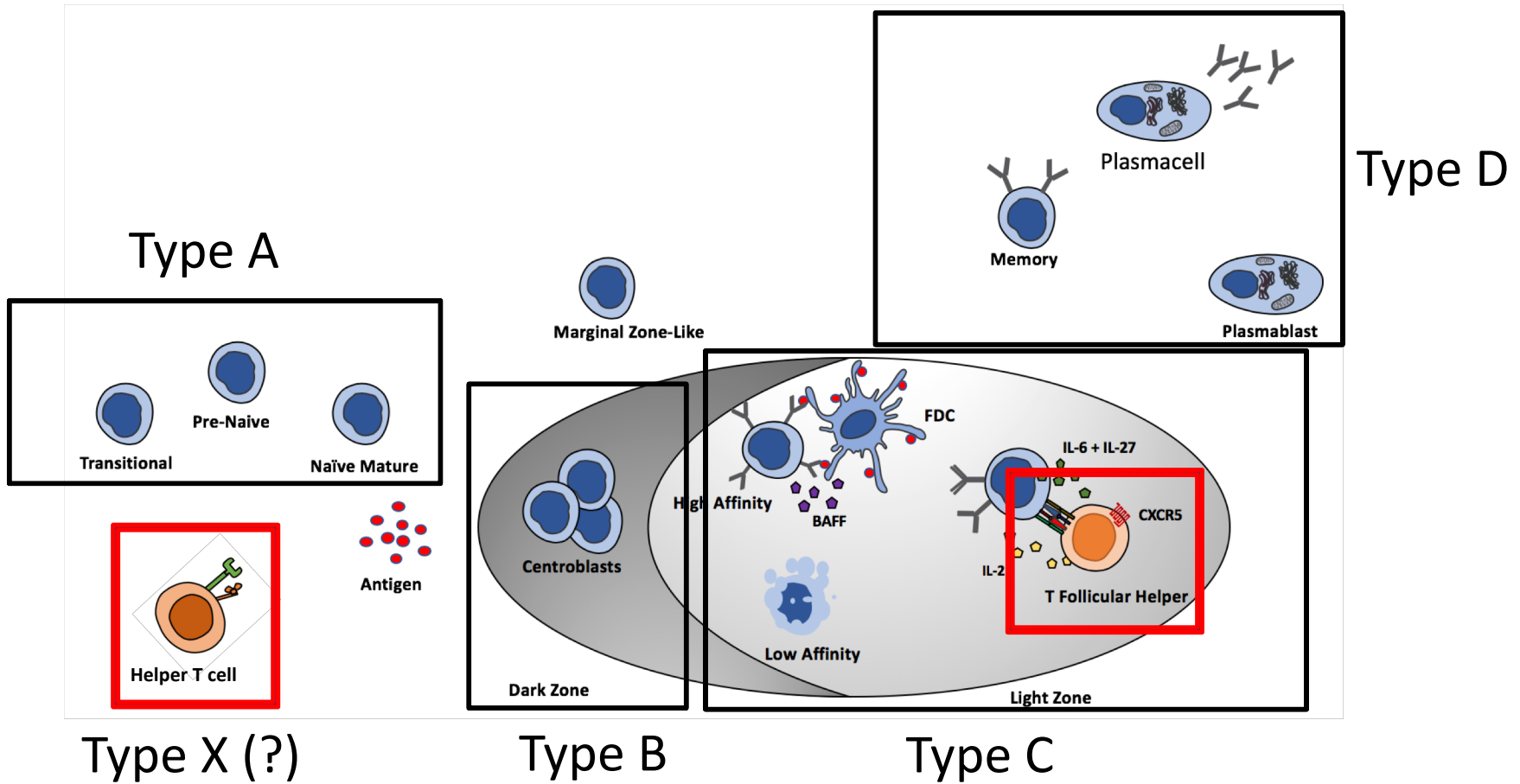


Why use flowcytometry as a first step in the diagnostics of PID?



1. Application of flow cytometry in SCID
2. Current application of flow cytometry in CVID
3. Future applications of flow cytometry in CVID

Defective B lymphocyte maturation in CVID



Defective B lymphocyte maturation in CVID

