Diagnosis for Primary Immunodeficiency (PID) by accessing available resources in South Africa

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The ICORD mission is to improve the welfare of patients with rare diseases and their families worldwide through better knowledge, research, care, information, education and awareness.

advocacy and engagement between those with ability to prevent, intervene, treat and provide supportive care for patients and families affected by rare diseases living in South Africa.

RARE DISEASES CONFERENCE 2016
Cape Town, South Africa | 19-22 October 2016
OVERVIEW

Your aims and mine

• Primary Immunodeficiency (PID) – really rare?
• Value of a diagnosis
• Diagnostic Criteria of PID
• How do/did we diagnose PID?
• Work of the SA PID Registry
• The “Cost” of essential diagnoses – Cases
• Access to PID genetic diagnosis
• Genetic Molecular capacity in Africa
What is PI?

People living with PI have an immune system that is not working correctly. But that doesn't mean they can't still live a full and active life.
Inborn errors of immunity are:

NOT just confined to a few rare, familial, monogenic, recessive traits impairing the development or function of one or several leukocyte subsets - resulting in **multiple, recurrent, opportunistic, and fatal infections in infancy**.

Appears that most individuals:

Each suffer from **at least one of a multitude of primary immunodeficiencies**, the dissection of which is helping to improve human medicine while describing immunity in natura.

**OVERALL**: 1 in 1200 livebirths
RARE Diseases – “widely spaced”

- Europe: rare when it affects 1 person per 2000.

- Depends on the degree of specificity used when classifying the different entities/disorders.

- Nearly all genetic diseases are rare diseases, but not all rare diseases are genetic diseases.

- Serious, often chronic and progressive, diseases, over 50% appear during adulthood.

- No cure for most?, but appropriate treatment and medical care can improve quality of life.

- Suffer from a deficit of medical and scientific knowledge: Difficulties in their quest for a diagnosis.

- Science can provide some answers: Hundreds of rare diseases now diagnosed through a biological sample test. And knowledge of the natural history of these diseases is improved by the creation of registries and research networks.
The evolving range of PID as we live longer...

• Nearly 300 monogenic traits

• Single type of infection predisposition also

• Any severe infectious illness potential PID

• Polygenic inheritance patterns

PID Diagnosis masked until times of medical progress!
Discovery of Major Types of PI

- 1922 Neutropenia
- 1926 Ataxia-Telangiectasia
- 1929 Chronic mucocutaneous candidiasis
- 1937 Wiskott-(Aldrich) syndrome
- 1944 Purification of γ-globulin
- 1950 Lymphocytophthisis (SCID)
- 1952 Agammaglobulinemia (XLA) and treatment with γ-globulin (1993 Identification of Btk as site of mutation in XLA)
- 1953 Alymphocytosis (SCID)
- 1954 Acquired agammaglobulinemia in an adult woman (CVID)
- 1957 Chronic granulomatous disease
- 1957 Swiss-type agammaglobulinemia and lymphopenia (SCID)
- 1968 Bone marrow transplantation for SCID
• 1982 Recognition of AIDS
• 1982 IVIG in the US
• 1991 Gene therapy trials for ADA deficiency

Ongoing – GENE PANELS and new PID genes

“Invitae Announces Major Expansion of Its Test Menu for Neurological, Pediatric, and Rare Genetic Conditions and Introduces New Panels for Inherited Metabolic Disorders and Newborn Screening Confirmation “

(Company achieves mid-year goal of more than 1,000 genes in production)
Evolving IUIS Classification (2014)

• predominant antibody deficiencies,
• combined T-cell and B-cell immunodeficiencies,
• other well defined immunodeficiency syndromes,
• congenital defects of number and/or function of phagocytes,
• complement deficiencies,
• defects of immune dysregulation,
• autoinflammatory disorders,
• defects in innate immunity
• Phenocopies

ASSISTED by
ESID Clinical Diagnostic Criteria 2016
Who will **NOT** develop PID?
Diagnosis

Diagnostics: The art or practice of medical diagnosis

• Symptom or a distinguishing feature serving as supporting evidence in a diagnosis.

• An instrument or a technique used in medical diagnosis.
Value of Diagnostic

• “The value of a diagnostic test is not simply measured by its accuracy, but depends on how it affects patient health”

• Improvements in test accuracy will not benefit patients unless they lead to changes in diagnoses and patient management

• Improved decision making is only one route by which tests affect patient health - empirical evaluations are needed to compare the effect of test strategies on patient health
Bottom line of diagnosis

1. Patient given diagnostic test
2. Test result produced
3. Diagnosis made
4. Management decided
5. Treatment implemented

Patient outcome
Symptom diagnostic **JMF WARNING SIGNS**

**10 Warning Signs of Primary Immunodeficiency**

Primary Immunodeficiency (PI) causes children and adults to have infections that come back frequently or are unusually hard to cure. 1/500 persons are affected by one of the known Primary Immunodeficiencies. If you or someone you know is affected by two or more of the following Warning Signs, speak to a physician about the possible presence of an underlying Primary Immunodeficiency:

1. Fat or May have air infections within one year.
2. Two or three serious skin infections within one year.
3. Two or three months an infection with like arthritis.
4. Two or three pneumonia within one year.
5. Fie red or grey skin growths.
6. Fat in the child's leg.
7. Skin infections.
8. Need for numerous antibiotics to clear infections.
9. Test of the skin, lack of hair.
10. A family history of PI.

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Modified for South Africa

+ BCG dissemination
Recurrent TB
Meningococcal Infection
“Local” Warning signs for primary immunodeficiency (Modified Modell)

• Eight (6) or more new ear infections within 1 year.
• Two or more serious sinus infections within 1 year.
• Two or more months on antibiotics with little effect.
• Two or more pneumonias within 1 year.
• Failure of an infant to gain weight or grow normally.
• Recurrent, deep skin or organ abscesses.
• Persistent thrush in mouth or elsewhere on skin, after age 1.
• Need for intravenous antibiotics to clear infections.
• Two or more deep-seated infections.
• Parasitoses (e.g. PJP and Giardia).
• Auto-immune manifestations, especially in the very young.
• A family history of Primary Immunodeficiency (or unexplained early death).

And

• BCG Dissemination
• AND Recurrent Meningococcal Infections
• Recurrent tuberculosis
Strongest Predictors for PID

• **Family History** – the most important predictor of PID

- **Use of IV antibiotics** for sepsis and **failing to thrive** for neutrophil and T cell related disorders

- **Hypocalcemia** with or without seizures, **congenital heart defects** (mainly conotruncal anomalies), **absence of thymic** shadow on CXR, **delayed umbilical cord** detachment (>30 days)
CLINICAL ALGORITHM DIAGNOSIS

Bousfiiha, Journal of Clin Imm 2013, 33(6)1078-1087
OR : Step wise laboratory testing - STAGE 1 TESTING

Test for HIV, CMV, EBV, TB where relevant

FBC & differential count: **Number of PN (<500/mm3)**

Lymphocytes < **1500**

CRP & ESR

**Signature organisms eg** BCG, PCP, Meningococcus

Screen for Cystic Fibrosis where indicated (SWEAT TEST)

Quantitative Immunoglobulins: IgG, M, A & E or **globulin fraction.**

IgG value of less than 3 g/L (300 mg/dL) – Cheap Rule Out
And some have **this testing** available (?)

Genetic defects of TLR/IL-1R signaling pathways (eg Herpes Simplex Encephalitis)

MSMD: MSMD-causing gene products in the IL-12/23-IFN-γ circuit. (eg Mendelian susceptibility to TB)
PIIDs: Diagnostic Procedure

Clinical
- Clinical phenotype, physical examination
- Origin, family history

Laboratory
- STAGE 1 Testing
- Immunophenotyping
- Functional assays (+/- specific of the suspected PID)

Genetic
- Genetic diagnosis
“A national registry for PID helps to describe the locally reported spectrum of genetic immune deficiency diseases. In that way, it addresses the clinical care needs of patients, the services needed, the monitoring requirements of treatment, training requirements and also locally relevant research questions. The added benefit is the creation of awareness; the ultimate aim is to improve the care of PID patients.”
Presenting Symptoms

- Respiratory: 50%
- Skin infections: 11%
- Fever / pyrexia: 16%
- GIT related: 7%
- Body Swelling: 3%
- CNS infection: 4%
- Septicaemia: 2%
- Bone and Joint: 1%
- Asymptomatic: 6%
Clue to PID - History

Family History of PID

- 42% Unknown
- 29% Positive
- 29% Negative
315 Patients recorded of 5800 min expected

IUIS Diagnostic Categories

Number of Cases

- Combined ID (CD)
- Syndromes (S)
- Antibody (A)
- Dysregulation (D)
- Phagocyte Defect (P)
- Innate Defects (I)
- Autoinflammatory (AI)
- Complement Def (C)
- Unidentified ID (UID)

Counts:
- Combined ID (CD): 20
- Syndromes (S): 18
- Antibody (A): 145
- Dysregulation (D): 2
- Phagocyte Defect (P): 21
- Innate Defects (I): 7
- Autoinflammatory (AI): 10
- Complement Def (C): 88
- Unidentified ID (UID): 4
Local relevance: TB findings

- 281 patients - **15 patients with 28 episodes of TB**
  
  (3 co-infections with *Mycobacterium avium* (*M. avium*) )

- 11 Severe Combined Immunodeficiency (SCID) - 5 known episodes of BCG dissemination
- 7 MSMD suspected or confirmed/ very likely (2 NEMO def excluded)
- Mycobacterial infections - 2 patients with Agammaglobulinaemia,
  
  - 2 patients with CVID
  - 2 NEMO deficiency (1 and 3 episodes of TB episodes respectively)
- 1 patient with Interferonopathy – MDR, Spinal TB
- 1 persistent CNS BCGosis – PID –novel mutation MAP3K
Diagnostic PID Test Cost

**BASIC PID SCREEN:**

- **FBC&Diff** – R 200.00
- **IgG,M,A** - R 373.00
- **HIV Elisa** - R 200.00

**TOTAL : < R 800.00**
Cost of conventional diagnostic investigation $ vs “yield” of diagnosis

Simple........ diagnoses

**Combined ID**
- “Typical” SCID R 600.00 (43 US $)
- With Syndromes
  - Hyper IgE – “classical” R 200.00 (14$ US )

**Antibody related**
- Agammaglobulinaemia
- (Transient) Hypogammaglobulinaemia R 400.00 (29 US $)
- IgA – true symptomatic deficiency

**Phagocyte**
- Persistent Neutropaenia
- Classical Chronic Granulomatous Disease R 200.00 (~ R2500)

**Complement**
- C5/C6 Complement deficiencies
- HAE Type I
But Diagnostic Cost $$$$$$ - if not so simple.........

• Hypomorphic/leaky SCID
• Evolving Syndromic features
• Hypogammaglobulinaemias
• Prolonged transient HGG
• CGD
• Dysregulations
• Autoinflammatory
• Phenocopies

THE COST OF GENOMICS.........?
4 Pillars of PID Diagnosis – which Shoe Fits?

History
Examination
Stage 1 laboratory (and extended Immune phenotyping)
Genetics
Genetic Diagnosis
Orphanet Directory of diagnostic tests

Intended to help professionals to obtain:

a) timely and

b) accurate diagnosis for patients affected by a rare disease

("Data Collection and update of diagnostic tests" procedure, soon available)

Diagnostic test in Orphanet is tagged:

a) one technical procedure of 3 levels
   i) Speciality: main method cat. (eg Mol Gen)
   ii) Objective: goal of test (eg Target Mut)
   iii) Technique: spec. technology (eg Sanger)
Implications for clinical genetic testing

Limitations as a diagnostic test:
• High ‘analytical specificity’
• Often low ‘clinical sensitivity’

→ Phenotype-based approach to diagnosis of PIDs:
  • Genetic testing only available, or useful, in certain instances
Phenotype-based approach is limited

• Only a minority of monogenic disorders have been addressed by these methods

• Many genetic disorders don’t have unique phenotypes

• **Genetic heterogeneity:**
  • Individuals with similar phenotypes may results from a mutation in any one of many genes

• Multifactorial disorders:
  • Person’s phenotype has multiple genetic (and environmental and epigenetic) contributions
Because tests can affect patient health by:

• changing treatment decisions
• affecting time to treatment
• modifying patient perceptions and behaviour
Example
The Newborn Screening (NBS) for PID

• **TRECS** (T cell receptor excision circles) & **KRECS** (Kappa-deleting recombination excision circles)

• Analysis of **SCID screening** results in over 3 million infants from 11 programs of population-based NBS with the TREC: Fifty-two cases of SCID and leaky SCID/Omenn syndrome, incidence of 1 in 58,000 births

• Goal of NBS: **detect treatable disorders** that are threatening to life or long-term health before they become symptomatic, ie PID

• **SCID only one of several (PIDs)** presenting early in life. Not completely sensitive, but NBS test for SCID able to identify some other PID with profound reduction of naïve T cells e.g. complete DiGeorge syndrome, leaky SCID, ataxia telangiectasia but also in Prematurity.

• Awareness of **genetic heterogeneity**, (i.e. the situation when a single phenotype could be caused by any one of multiple alleles or non-allelic different locus mutations)

• “Poor man’s alternative”: **CD3 T cell count of ≤1500 cells/microL and** absence of naïve T cells (oligoclonality)
Case
EMERGENCY

Male infant
Evolution over many months...

- Recurrent infections
- Intractable diarrhoea
- Eczematous rash
- Multiple auto-antibodies
Astute clinician suspects IPEX syndrome

- Immune dysregulation
- Polyendocrinopathy
- Enteropathy
- X-linked recessive

IPEX: requires urgent BMT!
<table>
<thead>
<tr>
<th>Result:</th>
<th>Hemizygous mutation identified</th>
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</table>
| Mutation details: | Gene: FOXP3  
Location: Exon 12  
DNA Description: c.1157G>A  
Protein Description: p.Arg386His (p.R386H)  
Consequence: Missense |

Interpretation

is hemizygous for a previously reported FOXP3 missense mutation, p.R386H (Tsuda et al 2010 J Autoimmun 35:265-268). This mutation is predicted to be pathogenic and the result confirms a diagnosis of IPEX syndrome.

→ Urgent result allowed for successful stem cell transplant
**Orphanet**

**Rare diseases are rare, but rare disease patients are numerous**

<table>
<thead>
<tr>
<th>Rare diseases</th>
<th>Orphan drugs</th>
<th>Expert centres</th>
<th>Diagnostic tests</th>
<th>Research and Trials</th>
<th>Patient organisations</th>
<th>Professionals and institutions</th>
<th>Other information</th>
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<tr>
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</table>

**Search**

- **Search by sign**
- **Classifications**
- **Genes**
- **Encyclopedia for patients**
- **Encyclopedia for professionals**
- **Emergency guidelines**

**Simple Search**

- **Disease name**
  - Gene name or symbol
  - OMIM
  - ICID
  - Orpha number

**Immune dysregulation-polyendocrinopathy-enteropathy-X-linked syndrome**

<table>
<thead>
<tr>
<th>Orpha number</th>
<th>Synonym(s)</th>
<th>Prevalence</th>
<th>Inheritance</th>
<th>Age of onset</th>
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<tr>
<td>ORPHA3042</td>
<td>Autoimmune enteropathy type 1</td>
<td>1/1 000 000</td>
<td>X-linked recessive</td>
<td>Infant, Neonatal</td>
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**Summary**

**Immune dysregulation - polyendocrinopathy - enteropathy - X-linked (IPEX) syndrome** is a severe congenital systemic autoimmune disease characterized by refractory diarrhea, endocrinopathies, cutaneous involvement, and infections.

Prevalence is unknown. Less than 150 cases have been reported to date but the disease has probably been underestimated.

IPEX syndrome usually develops during the first few days or weeks of life and affects exclusively boys. It manifests with the sequential appearance of the triad of enteropathy, autoimmune disease, and cutaneous involvement, but the clinical features and severity of the disease can vary considerably between individuals. Severe autoimmune enteropathy manifests with intractable secretory diarrhea leading to malabsorption, electrolyte disturbance and failure to thrive. Vomiting, itus, gastritis or colitis can also be observed. Patients also present with autoimmune endocrinopathies, generally insulin-dependent diabetes mellitus (type 1 DM), but also thyrotoxicosis leading to hyperthyroidism or hypothyroidism. Skin involvement consists of a generalized pruriginous eruption resembling eczema, prurigo, and/or atopic or exfoliative dermatitis. Less frequently,
Case of mistaken “neglected child”
Severely underweight, late onset severe bacterial, viral, fungal infections...gradual onset vitiligo, enteropathy, encephalopathy
LRBA Deficiency CVID
(common variable immunodeficiency variant) on exome sequencing

LRBA: Lipopolysaccharide (LPS)-responsive beige-like anchor (LRBA)- novel gene essential for normal function of the immune system (vesicle trafficking).

Eighth CVID gene, mutation of which causes CVID and autoimmunity, and is associated with inflammation.

Treatment potential – but too late in this case.
A family with agammaglobulinaemia

CASE Counselling

WHY Do They Die?

(HEPATITIS early Adulthood)

• “Poor” SES
• All lived in one house
• Back yard Wendy houses
• 1 toilet inside house
• Bucket toilets

• Adopted as baby
• Middle class family
• At 31 years now:
  • Employed
  • Healthy
  • Good quality of life

(Slide M Urban)

Mother: Not accepted diagnosis
Case
Severe Infections but **normal immune tests**!

- Neonatal Erythroderma
- Infancy Septic Pericarditis
- Young Boy Tuberculosis
- Teenager M. Avium arthritis
Later Ectodermal Dysplasia

**NEMO deficiency**

*Nuclear factor-kappa B essential modulator*/*Inhibitor of Kappa B Kinase gamma (IKK gamma)* protein is required for the activation of the NF-kappa B family of transcription factors, regulate gene expression and the development of a number of organ systems, including the immune system, alerting to any type of infection.
Need for access and feasibility of molecular diagnosis

• **Confirmation** of defined clinical phenotype
• Where overt PID **immunophenotype is lacking**
• Diagnosis for PID **phenotype diversity**
• Diagnosis for PID “**non classical**” eg. involvement of non-hematopoietic cells such as in ectodermal dysplasia ID with uncertain immune mechanisms causing recurrent infections
• Diagnosis not only of “**public**” genes – non-redundant -required for protective immunity to multiple microbes but also “**private**” genes conferring specific immunity to one pathogen

Cost effectiveness  data analysis  team discussion
Public Genes: Populations studied in the AGVP

Novel evidence of complex, regionally distinct hunter-gatherer and Eurasian admixture across sub-Saharan Africa.

Identified new loci under selection, including loci related to malaria susceptibility and hypertension....
Molecular **Diagnostic** Feasibility in Developing countries
Ready for genomics?
Need for the Collaboration......
Limited resources of genome sequencing in developing countries: Challenges and solutions

“The situation remains unaltered in several regions of the world, especially Africa”

Recommendations:
increasing research funding,
establishing centers of excellences,
encouraging international collaborations and
organizing specialized training programs as possible potential solutions for

Sustainable future improvement of genomic research in developing countries
PIDDGEN
Primary Immunodeficiency Diseases Genetic Network

• AIM: identification of novel candidate genes for susceptibility to tuberculosis in PID.
• Scope of Project: to develop genetic screening tools for PID patients.

Faculty of Health Sciences, University of Stellenbosch & National Health Laboratory Service (NHLS) Tygerberg
TAPER™DATA – Analysis

- TAPER™ is a seven tier ‘pipeline’ for data filtration (B Glanzmann)
- Which variants require further assessment?
## Bioinformatic filtering using TAPER

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<thead>
<tr>
<th>Description</th>
<th>Database</th>
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<tr>
<td>TOTAL GENETIC VARIANTS</td>
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<td>Remove synonymous and non-frameshifts</td>
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<tr>
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These variants require further assessment
The journey continues
New Frontiers in Immunology

• **clinical genetics, epidemiological genetics, and evolutionary genetics**, with the definition of PIDs in this context, constitutes a new frontier in immunology.

• **connect basic research with patient care** From the knowledge gained through this long and enlightening journey through the history of our planet and the evolution of our race, and for the discoveries yet to come the future looks promising.

• **to provide better treatment and in some case even the hope of cure** to our patients. “

"These patients, in fact, often are not given enough attention because the idea of profit prevails over the value of human life.

It is fundamentally important to promote greater empathy in society, so that nobody remains indifferent to our neighbour's cry for help, including when he or she is suffering from a rare disease."
Come JOIN us
Acknowledgements

- **PIDDGEN TEAM**

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- Ms Nikola Schlechter
References

The South African PID Journey and the Future

- David Beatty – dedicated PID service and laboratory at RXH - 1983
- Patrick Bouic - Immunology Laboratory Tygerberg
- Paul Potter – Allergy and Clin Imm Service at GSH
- The South African Bone Marrow Registry -1991, BMT GSH
- Tygerberg – dedicated PID service - 1991
- PiNSA Joy Rosario - 2001 (assistance of IPOPI)
- PIDDSA - 2006
- PID Registry - 2009
- South African Immunology Society Congress -2009
- African Society for Immunodeficiency Diseases -2009
- BMT Gauteng private - 2011
- PID Service Pretoria Academic -2016
- PID Service Albert Luthuli – 2016
- The Future – Collaboration between Industry- Private Enterprise-Universities