NEWSPID

NEWSLETTER from IS**PID**
Indian Society for Primary Immune Deficiency

JAN – JUNE 2018

Message from the editor

Hello everyone!

ISPID is a year older and is more than 100 members strong now.

ISPID national conference in March 2018 has set in motion what promises to be action packed year ahead (checkout the events calendar on page 6). PIDCON 2018 was held at Jaipur under the organizational capacity of Drs. V.M. Katoch and Ashok Gupta. The conference brought together clinical immunology colleagues and basic scientists under the same roof as pediatricians, hematologists, dermatologists, geneticists and transplant physicians thus providing a one-stop-shop for all discussions pertaining to primary immunodeficiencies. A postgraduate quiz was organized for the first time under the banner of ISPID during this conference.

The year also has witnessed a lot of initiatives from ISPID. PID awareness CMEs were conducted across the country. There is brisk work in progress to develop uniform diagnostic criteria and reporting formats.

In this edition, the we focus on BCGosis, a manifestation of many severe primary immune deficiencies. BCGosis is often difficult to diagnose as it is not thought of commonly in routine clinical settings and even difficult to evaluate as the investigations are not available easily. We hope you find the discussion useful.

Best regards,

Nita

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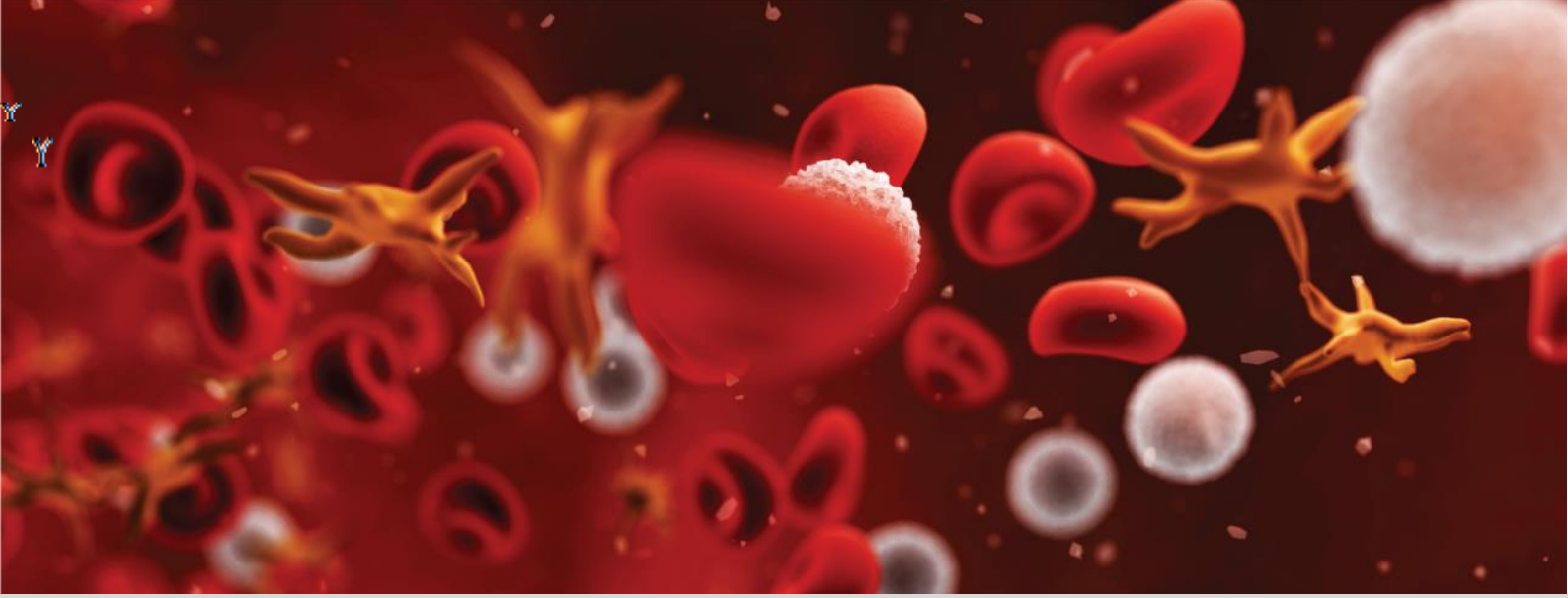

Case of the month

Clinical discussant
Dr Mukesh Desai

Laboratory discussant
Dr Manisha Madkaikar

Centers
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CASE

A 6-month old girl, 1st by birth order, born of non-consanguineous marriage was born normally and received BCG vaccination on day 2 of Life. She presented at day 15 of life to a nearby hospital with persistent left axillary abscess and fever. She was admitted there for 15 days where the abscess was drained.

She presented again at 3 ½ months of age with complaints of fever, cough, coryza, generalized oedema, breathlessness and petechial rash on upper and lower limbs. Complete blood count revealed anaemia (Hb-6.7gm/dl) and leucocytosis (TLC-43800/µl; P:41%, L:52%, M:07%, E:0%) and thrombocytopenia (platelet count: 34,000/µl). Ultra-sonogram of left axilla revealed multiple enlarged lymph nodes. Pus was aspirated from one of the nodes which grew Pseudomonas aeruginosa and she was treated with sensitive antibiotics. She also received packed red cell transfusion for anaemia. Serum ferritin and triglyceride levels were high with fibrinogen was low. Serum immunoglobulin estimation revealed elevated IgG levels. USG abdomen revealed mild hepatosplenomegaly with hypoechoic lesions in liver suggesting chronic bacterial, mycobacterial or fungal infection. IgM CMV antibody was positive. Bone marrow aspiration and biopsy was done, which showed evidence of hemophagocytosis with toxic changes in myeloid series.

In view of fever, mild hepatosplenomegaly, haemophagocytosis on bone marrow aspirate, and biochemical parameters consistent with haemophagocytic lymphohistiocytosis (HLH) she was started on treatment for HLH with dexamethasone & cyclosporine along with intravenous ganciclovir. She required multiple blood transfusions during her hospital stay in view of persistent anaemia and thrombocytopenia. She recovered from this episode and was discharged. She was continued on HLH protocol for the next 8 weeks.

At the age of 6 months, there was recurrence of abscess in the left axillary lymph node with ulcerative skin lesions on the scalp. Pus drained from the lymph node and skin lesions was positive for AFB. In view of disseminated TB at the 6 months of age she was referred to our centre for further evaluation.

Laboratory findings at our centre revealed a white blood cell (WBC) count of 25.01×10^9/L with absolute lymphocyte count of 9.930×10^9/L and absolute neutrophil count of 12.11×10^9/L. Haemoglobin was 8.3g/dl and platelet count was 216×10^9/L. Flow cytometric evaluation of lymphocyte subsets showed elevated absolute counts of Natural Killer (NK) cells, cytotoxic T-cells with normal T-helper and B-cells.

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Sr. No.	Lymphocyte Subpopulation	Lymphocyte%	Absolute Count /mm3	Normal Absolute Count /mm^3
2	$CD19^+$ B lymphocytes	8	800	**610 – 2600**
4	$CD3^+$ T lymphocytes	66	6603	**1900 – 5900**
5	$CD3^+$/ $CD4^+$ Th lymphocytes	20	2001	**1400 – 4300**
6	$CD3^+$/ $CD8^+$ Tc lymphocytes	33	3301	**500 – 1700**
7	**$CD3^-$/ $CD16^+56^+$ NK Cells**	**24**	**2401**	**160 – 950**

Perforin expression and granule release assay was normal. Nitro blue tetrazolium (NBT) test was normal. Serum immunoglobulin estimation revealed elevated IgG levels (IgG- 2095mg/dl, IgA- 71 mg/dl, IgM- 245 mg/dl). She was evaluated for Mendelian Susceptibility for Mycobacterial Diseases as the preliminary immunological evaluation was non-conclusive.

MSMD work revealed enhanced expression of IFNγR1 on monocytes compared to normal control (more than 7-fold increase) tested using CD119 antibody (eBioscience, GIR-208, PE conjugated) by flow cytometry. pSTAT1 (BD Biosciences) expression on IFNγ stimulated monocytes was also found to be impaired. Serum IFNγ level using ELISA were within normal range. Molecular analysis by Sanger sequencing of the IFNγ receptor 1 gene showed frame shift with deletion of 4bp (TAAT)[c819_822del TAATp.] in heterozygous state in exon 6. No mutation was detected in both the parents at the same position.

Child was started on anti-tuberculous treatment. She responded well to therapy and is presently doing well.

DISCUSSION

Q1: What is the difference between BCGitis and BCGosis?

A: The Bacille Calmette-Guérin (BCG) is an attenuated strain of *Mycobacterium bovis* which is is widely used for vaccination against tuberculosis especially in the paediatric age group. World Health Organization (WHO) recommends that all infants in highly endemic countries receive a single dose of the BCG vaccine.

BCG vaccine is considered safe in immunocompetent children. However, infection, even disseminated infection, caused by BCG has occasionally been reported. The incidence of BCG infection is approximately 1:10,000–1:1,000,000. The BCG-induced disease phenotypes were designated as local, regional, distant, or disseminated pattern based on a revised paediatric classification proposed by Hesseling et al. Most common complications include purulent regional lymphadenitis of the ipsilateral nodes and ulceration and swelling at the injection site, also known as BCGitis. Distant and disseminated BCG infection (known as BCGosis) is a rare but potentially dangerous complication of BCG vaccine which almost always occurs in children with immunodeficiency. The frequency of BCGosis is estimated to be 0.59 per 1 million vaccinated children.

Q2: When do you suspect BCGosis in a child? How do you proceed to confirm your diagnosis? What are the investigations done to assess the extent of the dissemination

A: Patients with a positive history of inoculation of BCG vaccine and two or more signs and symptoms of a systemic syndrome compatible with mycobacterial disease including: fever, weight loss, lymphadenopathy or cutaneous abscesses, pneumonia, osteomyelitis and hepatosplenomegaly should raise suspicion regarding BCGosis. Diagnosis of BCGosis is confirmed by demonstration of AFB in lymph nodes aspirates and skin biopsies, typical histopathological features and specific polymerase chain reaction (PCR). Taking a good family history in such patients could be beneficial and might lead to a timely diagnosis, which in its turn would result in early intensive treatment and could be life-saving.

To assess the extent of the dissemination evidence of BCG infection including a histopathological demonstration of acid-fast bacilli at two or more anatomic sites far from the region of vaccination such as lymph nodes or cutaneous abscesses outside the region of inoculation, liver biopsy, gastric aspiration and bone marrow aspiration are needed.

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Q3: BCGosis or disseminated BCG infection always occurs in patients with immunodeficiency. What are the primary immunodeficiencies that present with disseminated BCG infection?

A: Patients with severe complications to BCG vaccination should be evaluated for underlying immunodeficiency; either primary or secondary. Generally, BCG vaccines are considered safe in immunocompetent hosts, however sometimes it causes complications in the form of lymphadenitis, fistula formation and rarely disseminated disease and death. BCGosis is associated with primary immunodeficiencies such as combined immunodeficiencies (Severe Combined Immuno Deficiency [SCID], Hyper IgM syndrome etc.), chronic granulomatous disease and Mendelian Susceptibility to Mycobacterial Diseases (inherited defects in IL-12 IFN gamma axis).

The severity and the onset of BCG related complications in various PIDs is different. SCID patients usually present with disseminated BCG and failure to thrive early in life. MSMD patients on the other hand, are relatively well children, do not have failure to thrive but can present with disseminated BCG infection. It has been reported that in SCID and MSMD patients, the BCG complications occur earlier and are often more severe than other PIDs. Both molecular (e.g., genetic form of PIDs) and vaccine-associated factors (e.g., BCG strain, age at vaccination) might influence the type of outcome after BCG vaccination. For CGD, most of the patients are prone to exhibit localized BCG lymphadenitis, although disseminated cases have also been reported.

In comparison with SCID and MSMD patients, the CGD patients usually clear the BCG infection. X-linked Hyper IgM and Hyper IgE syndromes are also other PIDs where BCGosis has been reported.

Q4: How would you manage BCGosis in a suspected immunodeficient child?

A. In SCID babies who have received BCG vaccination already, anti-TB treatment with INH and RMP should be initiated and escalated to four drugs if inadequate response or no progression is observed.

For BCGosis, anti-tuberculosis treatment including four or more anti-TB drugs, as per sensitivity, duration depends on clinical response and immune reconstitution of the patient.
Drug choice should preferably include a macrolide in view of atypical mycobacteria and depends on the BCG strain in use in the country. In India, Danish 1331 strain is used, hence it's better to include macrolide.

Duration of treatment:
In CGD or MSMD it might range up to 2 years while for patient for SCID it would be till post-transplant immune recovery. Then, a prophylactic programme with two drugs should be continued, until complete immunological reconstitution after HSCT is achieved. Some studies show that along with anti-TB treatment, administration of rhIFN-γ provided better control of BCGosis/BCGitis. However, the results need to be verified by a large-sample, randomized, double-blind, placebo-controlled study.

Q5. In a child with primary immunodeficiency with BCGosis, how will you proceed with managing the primary immunodeficiency?

A.SCID is considered a medical emergency and irrespective of presence or absence of BCGitis, the child would need a bone marrow transplant as soon as possible. A younger age at transplant with fewer infections prior to transplant has a better prognosis. For children with CGD, bone marrow transplant is curative. In children with MSMD, bone marrow transplant has been attempted in severe defects with variable results.

Q6. Can you briefly describe a step wise evaluation for underlying primary immunodeficiency in patients with BCGosis?

A: It has been documented that a number of PIDs like severe combined immunodeficiency (SCID), chronic granulomatous disease (CGD) and Mendelian susceptibility to mycobacterial diseases (MSMD) are prone to BCGosis. In addition to these diseases, other PIDs such as hyper-immunoglobulin E syndrome, X-linked hyper IgM syndrome also have increased vulnerability to BCG infection.

Diagnosis

Mendelian susceptibility to mycobacterial diseases

Mendelian susceptibility to mycobacterial diseases (MSMD) is a rare form of primary immunodeficiency. These patients have increased susceptibility to salmonellosis, tuberculosis, and viruses [(cytomegalovirus virus [CMV], human herpesvirus 8 (HHV8)] and weakly virulent nontuberculous mycobacteria (NTM) including BCG.

Genetic defects for MSMD have been identified in the interleukin-12–IL-23–interferon-γ (IFNγ) signalling pathway. Mutations have been identified in the genes encoding IFNγ receptor 1 (*IFNGR1*), IFNγ receptor 2 (*IFNGR2*), STAT1 (*STAT1*), the p40 subunit of IL-12 and IL-23 (*IL12B*), IL-12 receptor β1 (*IL12Rβ1*) and nuclear factor-κB (NF-κB). Recently, mutations in *CYBB, ISG15* and *IRF8* have also been described as responsible to cause MSMD. These patients have a vast heterogeneity in clinical manifestations and in their susceptibility to mycobacterial infections. Patients with complete IFNγR1 or -R2 deficiencies have higher mortality rate and present in early childhood with disseminated mycobacterial infections caused either by BCG or NTM. However, patients with other deficiencies such as partial IFNγR1, complete IL-12B and IL-12 receptor have milder clinical manifestations and usually present at later age. IL12Rβ1 deficiency has been reported as the most common form of MSMD.

Initial evaluation includes complete blood count (CBC) to rule out lymphopenia and neutropenia. HIV is ruled out in all cases. Lymphopenia if seen is further evaluated by lymphocyte subset analysis to rule out SCID. Nitro-blue-tetrazolium test and dihydrorhodamine test is used for diagnosis of CGD. Serum immunoglobulin estimation helps in SCID and Hyper IgM patients. If these tests are normal, then the patient is further evaluated for MSMD. MSMD evaluation involves flow cytometric evaluation of IFNγR1(CD119) and IFNγR2 expressions on monocytes for diagnosis of IFNγR1 and IFNγR2 deficiency respectively and CD212 expression of on activated T cells for IL-12Rβ1 deficiency. Functional assays like pSTAT1 and pSTAT4 expression after stimulation further validate the IFNγR1 and IFNγR2 deficiencies and IL-12Rβ1 deficiency respectively. Estimation of serum cytokines such as IFNγ and IL-12p40 by ELISA at baseline and after stimulation also helps in the diagnosis of MSMD.

Diagnosis is confirmed by identification of the molecular defect by Sanger sequencing of the causative gene. More advanced techniques like targeted next generation sequencing (NGS) or whole exome/ genome sequencing may be considered in patients with strong clinical suspicion of PID and in whom immunological evaluation is inconclusive.

Q7. What are the histopathological features of BCGosis? Do the histopathological features help us in understanding the severity of the underlying immunodeficiency?

A: In complete IFN-γR1 deficiency, the manifestation of lepromatous-like lesions following BCG immunization is suggestive of the absence of IFN-γR1 mediated immunity, whereas, tuberculoid granulomas almost definitely rule out complete IFN-γR1deficiency. Regardless of the underlying genetic etiology, appearances of NTM lesions are generally lepromatous-like. The microscopic pattern consists of 'lepromatous-like' inflammation or a mycobacterial spindle like tumor (MSP), can indicate that immunodeficiency is more likely to be severe. A shift from 'lepromatous' features to 'tuberculoid' features is correlated with a good prognosis. The prognosis of children with complete IFNγR deficiency is poor; it typically has severe, poorly delineated, multi-bacillary granulomas, with no epithelioid or giant cells and may lead to death because of overwhelming infection. Children with tuberculoid granulomas with well delineated, pauci-bacillary disease, epithelioid and giant cells and have a good outcome. The patients with partial IFN-γR deficiency due to mutations of either autosomal recessive or autosomal dominant IFN-γ receptor usually have less severe mycobacterial disease associated with tuberculoid granulomas.

Suggested reading

1. Norouzi S, Aghamohammadi A, Mamishi S, Rosenzweig SD, Rezaei N. Bacillus Calmette-Guérin (BCG) complications associated with primary immunodeficiency diseases. J Infect. 2012; 64: 543–554.

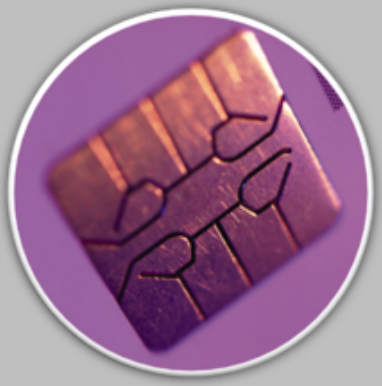
2. Shahmohammad S, Mohammad S, Mohammad R. BCGosis after BCG vaccination in immunocompromised children: Case series and review. J Peditatr Review. 2014; 2(1): 47-54.

5. Shrot S, Barkai G, Ben-Shlush A, Soudack M. BCGitis and BCGosis in children with primary immunodeficiency - imaging characteristics. Pediatr Radiol. 2016;46(2):237-45.

Journal Watch

1. Phenotype, penetrance, and treatment of 133 CTLA-4-insufficient individuals.
Schwab C et al. **J Allergy Clin Immunol**. 2018 https://doi.org/10.1016/j.jaci.2018.02.055.
CTLA4 mutation carriers presents with hypogammaglobulinemia, lymphoproliferation, autoimmune cytopenia, malignancies and respiratory, gastrointestinal or neurological features. CMV and EBV associated complications were monitored closely in many.

2. Long-term follow-up of IPEX syndrome patients after different therapeutic strategies: An international multicenter retrospective study. Barzaghi et al.
J Allergy Clin Immunol. 2018 https://doi.org/10.1016/j.jaci.2017.10.041
96 patients with genetically proven IPEX syndrome were analyzed retrospectively. When performed in patients with a low organ Involvement score, HSCT resulted in disease resolution with better quality of life, independent of age, donor source, or conditioning regimen.



Event Watch

National

1. **7th Introductory course on Primary Immunodeficiency Diseases**
Venue: IGMC, Shimla Date: 13 October 2018
Contact: ispid.contact@gmail.com
2. **The immunology of human tuberculosis: MSMD and beyond**
Venue: ITC Grand Central, Parel, Mumbai Date 14-15 October 2018
Contact: lifeatwarseries@gmail.com
3. **ISPID-Reach CMEs** at Agra, Patna, Shillong, Nagpur (June-Sept 2018)
Dates: TBD Contact: ispid.contact@gmail.com
4. **5th International Conference on Primary Immunodeficiency**
Venue: The Leela, Mumbai Date: 9-11 March 2019
Contact: ICPID2019@gmail.com

International

1. **ESID 2018- Meeting of the European Society of Immunodeficiencies**
Theme: PID from fetus to elderly
Venue: Lisbon, Portugal Date: 24-27 October 2017
Contact: https://esid.kenes.com/2018

Organizing team PIDCON 2018

Keynote Lecture
Dr Michael Lenardo
NIH, Bethesda

Indian PID perspective
Dr Surjit Singh
PGIMER, Chandigarh

We would love to hear from you.

Please send your comments, suggestions and cases/ images for discussion to ispidofficial@gmail.com

Editor, NEWSPID