Project: Jeffrey Modell Diagnostic Center

“A proposal for improving diagnostics and care of PID in Serbia”

Srdjan Pasic MD, Ph.D

Associate Professor in Pediatrics

Head, Dept. of Ped. Immunology

Mother & Child Health Institute of Serbia “Dr Vukan Cupic”

Belgrade, SERBIA

pasics@ikomline.net

phone + 381 11 3108 263

1. **History of Pediatric Immunology in Serbia and project background**

Primary immunodeficiencies comprise more than 150 genetically defined disorders. In Serbia, Department of Pediatric Immunology at the Mother and Child Health Institute, Belgrade is the leading center providing diagnosis and care for approximately 90% of pediatric patients with PID in Serbia.

Pediatric Immunology dept. at the Mother and Child Health Institute, Serbia, teaching tertiary-care pediatric hospital, was established in 1971 by late **Dr Mirko Mikuska**. He was trained in Boston, MS and New York, NY in an early 70's where he met the pioneers in pediatric immunology, Max D.Cooper and the late Robert A. Good. After his return home he diagnosed the first cases of CGD, WAS or antibody deficiencies. However, his major accomplishment was that he was able to share an idea with his collaborators what modern pediatric immunology should represent and what field it should cover.

Dr Mikuska passed away in 1989. **Dr Mario Abinun,** who joined Dr Mikuska in 1983**,** became Head of Dept. His primary interests were the long-term follow-up of the families affected with hereditary angioedema (HAE) or antibody deficiencies, including an introduction of C1-inhibitor treatment for HAE attacks and an introduction of IVIG for XLA or CVID in 1985 (see list of publications). By late 80's flow-cytometry became feasible in immunology labs in Belgrade, allowing more accurate diagnosis of severe combined ID (SCID) or the other combined PID. In 1990, the first haploidentical T-cell depleted (Campath-1M) stem cell transplantation in SCID was successfully performed by our team and shortly after, the second one SCT followed (M. Abinun, D.Lilic, S.Pasic, D.Vujic). In 1992, because of well-known tragic events in former Yugoslavia Dr Abinun decided to leave Yugoslavia. He gained the position of Consultant in Pediatric Immunology, NCL General Hospital, Supraregional centre for SCT in PID, Newcastle-upon Tyne, UK but he remained in close contact with us.

In 1991, **Dr Srdjan Pasic** started to work at Pediatric Immunology Dept. and increasing number of antibody deficiency (total N =90, excluding sIgA deficiency), severe combined immunodeficiency (N=20), WAS (N=12), CGD (N=19) or HAE (N=25) were diagnosed over the next two decades. In 1997, a new SCT unit at our Institute was opened but at that time it was possible to perform only HLA-identical SCT resulting in moderate number of SCT (N=5) while the other patients lacking a matched family donor were referred abroad. Beginning in the late 90's, Dr Pasic managed to improve both the diagnostics and treatment (incl. the first HLA-id SCT) in hemophagocytic lymphohistiocytosis (HLH), a fatal inherited condition. At present, we have more than 40 patients in our HLH registry with an overall survival of 50%. Also, in the past decade the first cases of hyper-IgM syndrome, Bloom syndrome, X-linked lymphoproliferative disease or Nijmegen breakage syndrome in Serbia were diagnosed (see list of publications).

Since 1997, **Day-Care Hospital** at our Institute represents the facility where both the follow-up and specialized care of PID patients, as well as, regular treatment with IVIG are performed.

Immunology laboratory, MCH Institute currently performs the following tests for diagnostics of PID: 1. immunoglobulin levels 2. concentrations of serum complement components including C1esterase function 3. PMA - stimulated NBT test 4.flow-cytometry with the use of basic monoclonal antibodies for diagnosis of PID and hematological malignancies (certified by Italian panel and European BFM panel).

Other immunologic investigations (IgG subclasses, lymphocyte proliferation studies, autoantibody screen, classic or alternative pathway of complement activation, etc.) are performed in other immunology labs. situated in Belgrade: Institute of Immunology, Medical School, University of Belgrade and Institute for transfusiology, Belgrade.

Genetic laboratory, M&CH Institute is performing FISH for 22q11 deletion syndrome and common mutation detection for Nijmegen breakage syndrome. Also, laboratory is equipped to perform functional investigations in DNA repair disorders. Since 2000, Team for Genetic Counseling, based at our Institute, was established and several prenatal diagnosis in PID families were performed, allowing the parents with previously affected child to have healthy children. Definitive analyses of chorionic villi samples for prenatal diagnosis were done in collaboration with Istituto Molecolare "A. Nocivelli", Brescia, Italy.

Serbian Patients Organization (SPwPID - Supporting persons with primary immunodeficiencies) is registered, non-profit organization, active for almost two decades, and it is recognized trough IPOPI. President of our patient's organization, Mrs. Dragana Koruga is well-established member of IPOPI Board and, even more important, qualified and dedicated person to our goal.

Other 4 pediatric tertiary care centers who occasionally diagnose children with PID are situated at cities of Belgrade, Novi Sad, Kragujevac and Nis. Some of their patients are referred to Mother and Child Health Institute for further evaluation.

Adult patients with PID, mainly with antibody deficiencies and HAE (N=40) are treated at Dept. for Clinical Immunology, University Clinical Centre of Serbia, Belgrade (Dr B. Bonaci, Dr S. Andrejevic) and in similar centers in Novi Sad (N=8). Also, 6 adult patients with antibody deficiencies, who were treated during childhood at M&CHI, currently are on regular IVIG substitution at the Clinical Centre "Bezanijska Kosa" (Dr.Z.Sporcic).

1.1. Project background

During the past decade, continuous medical education of pediatricians resulted both in better recognition and referral of patients for suspected PID. However, patients with PID are still not readily diagnosed or treated so that more basic education among pediatricians and primary health care physicians is needed.

More sophisticated diagnostic tools are also needed because the list of newly recognized PID became much longer. Also, precise diagnosis and specific disease-orientated treatment lead to a better chance for survival and an improved life-quality of PID patients.

Another important goal will be to motivate the patients and their parents trough the activities of the National Patients Organization aiming to increase the level of care of PID patients.

2. Proposal of project goals

Our future plans to improve diagnostics and treatment of PID include:

2.1. To increase the awareness about PID and their specific problems

- National Committee for Rare Diseases already included PID in the list of the diseases with specific needs and specific treatment modalities; further efforts should be made to include specific orphan drugs as regular treatment option for certain PID
- public campaign (media) during International Day for Rare Diseases (February) including meeting of the patients and their parents at our Institute
- education of pediatricians during traditional Pediatric Summer School or other annually held Pediatric Meetings
- education of primary care physicians trough meetings or PID workshop
- training for both pediatricians with an interest in pediatric immunology or laboratory-orientated doctors in developed laboratories abroad
- develop National Guidelines and Network for PID of 4 tertiary-care pediatric hospitals

2.2. To establish and maintain national PID Registry

- collect the data of all deceased and alive patients with the exact diagnosis of PID
- collect the data of all adult patients seen by internal medicine specialists
- make a link with once established National Registry with ESID database

2.3. To develop the existing or to introduce new diagnostic procedures

- the expanded panel of standard monoclonal antibodies for flow-cytometric assay
- specific antibody responses against protein or polysaccharide antigens
- flow-cytometry-based assays of intracellular protein expression

  (perforin, SAP, WASP, etc.)

2.4. **To educate the patients and the families trough National Patients Organization**

- organize educational summer camps
- organize local meetings with the patients and their families
- help decreasing the morbidity promoting a healthy life policy (nutrition, sport)
- help if specific problems or needs occur (social worker, team of psychologists, etc.)
- develop the booklets explaining the disease-specific problems

2.5. To improve the quality of treatment

- an introduction of subcutaneous scIg treatment for pts. with antibody deficiency
- availability of specific medications (e.g. ORPHAN drugs) for rare diseases

3. Milestones (Funding: JM Foundation and Octapharma support est. 45,000 USD per year)

A proposal of the activities during the first year of this project:

Education **Total:** **$ 18000**

- February, 2012 public campaign (media) during International Day for Rare Diseases including awareness for PID and special needs for treatment
- March 2012 - launching of educational workshops for pediatricians or general practice physicians (4 meetings per yr., an est. no of participants per 1 meeting 30) **$4000**
- March 2012 Education of one laboratory based immunologist for performing rapid protein based assays in diagnosis of PID in centers abroad 2 months **$2000**
- April, 2012 Education of **one/ or two** pediatric immunologist per year in specialized centers abroad for 2 months **$4000**
- July/ August, 2012 Summer Camp for PID patients; the booklet in Serbian explaining basic steps in prevention of infections in PID **$8000**

Patient registry **$9000**

- April , 2012 the Meeting of Pediatricians and Specialists of Internal medicine involved in care and treatment of both pediatric and adult PID patients: 5 teaching pediatric hospitals Belgrade (2), Novi Sad (1), Kragujevac (1), Nis (1) and 5 Internal Medicine Clinics affiliated with 4 Universities at before mentioned locations
- June, 2012 an Update of hospital-based Registry at Mother & Child Institute
- January - June, 2012 launching of National PID database at Mother and Child Health Institute (one administrator will be responsible for maintaining the database)
- September, 2012 link to the ESID registry with newly collected data (90 pts. were already included in the ESID Registry 2004-2005)

Laboratory requirements (the list of specific equipment and the reagents incl. cost enclosed)

- March, 2012 introduction of specific antibody measurement (ELISA)
- June, 2012 detection of intracellular proteins by flow-cytometry

Activities of National Patients Organization **Total: $ 6000**

- March, 2012 local meeting with the patients, Belgrade and Novi Sad
- May-June, 2012 local meeting with the patients, Kragujevac
- September, 2012 local meeting with the patients, Nis
- June, 2012 the booklet in Serbian explaining basic steps in prevention of infections in PID

Steps to improve the quality of treatment in PID

- 2012 a trial of subcutaneous Ig treatment for selected, small group of patients affected with antibody deficiency (XLA, CVID, etc.)

Laboratory equipment - 1st and 2nd year of the project, Immunology lab., MCHI, Belgrade (* 1st year of the project)

Total cost $ 12000

- CO_2 incubator **$6.500***
- Laminar air flow cabinet biosafety class II **2nd year**
- Microscope, inverted approx. **$ 3.000***

List of reagents (preliminary, to be adjusted according to suggested fundings) **$ 2500***

- Anti-XIAP clone 48 BD Cat. 610763 and anti-SAP. Abnova
- Anti CD132 gamma chain
- Anti CD25
- Anti CD163
- Anti IL-12Rβ1
- Anti CD15s
- Anti CD11a
- Anti CD18
- Anti FoxP3 antibody
- anti-WASP 3F3-A5 or anti-WASP B-9 (Santa Cruz Biotechnology, Santa Cruz, CA, USA)
- anti mouse PE- conjugated antibody
- PMA
- Ionomycin
- Anti CD69
- Anti perforin clone B-D48
- Brefeldin A
- Monensin

Total funding of the Project $ 45.000

The list of selected publications, Dept. of Pediatric Immunology, Mother & Child Health Ins.

1. **Pasic S**, **Abinun M**, Vlajic B, et al. Aspergillus osteomyelitis in chronic granulomatous disease: treatment with recombinant gamma-interferon and itraconazole. Ped J Infect Dis 1996; 15: 833-34.
2. **Pasic S**, Flannagan L, Cant AJ. Liposomal Amphotericin is safe in bone marrow transplantation for primary immunodeficiency. Bone Marrow Transplantation 1997; 19:1229-32.
3. **Pasic S**, Jankovic B, **Abinun M**, Kanjuh B. Intravenous immunoglobulin prophylaxis in an echovirus 6 and echovirus 4 nursery outbreak. Ped Infect Dis J 1997; 16:718-20.
4. **Pasic S**, Lilic D, Pejnovic N, et al. Disseminated BCG infection in a girl with the hyperimmunologlobulin-E syndrome. Acta Paediatrica 1998; 87:702-704.
5. Villa A, Bozzi F, Sobacchi C, Strina D, Fasth A, **Pasic S**, Notarangelo LD, Vezzoni P. Prenatal diagnosis of Rag-deficient Omenn syndrome. Prenatal Diagn 2000; 20:56-59.
6. Santagata S, Gomez CA, Sobacchi C, Bozzi F, **Abinun M**, **Pasic S**, Cortes P, Vezzoni P, Villa A. N-terminal RAG1 frameshift mutations in Omenns syndrome: internal methionine usage leads to partial VDJ recombination activity and reveals a fundamental role in vivo for N-terminal domains. Proc Natl Acad Sci USA 2000; 97:14572-77.
7. Villa A, Sobacchi C, Notarangelo LD, Bozzi F, **Abinun M**, Abrahamsen TG, Arkwright PD, Baniyash M, Brooks EG, Conley ME, Cortes P, Duse M, Fasth A, Filipovich AH, Infante AJ, Jones A, Mazzolari E, Muller SM, **Pasic S**, Rechavi G, Sacco MG, Santagata S, Shroeder ML, Strina D, Ugazio AG, Vallaho J, Vihinen M, Vogler LB, Ochs HD, Vezzoni P, Freidrich W, Schwartz K. V (D) J recombination defects in lymphocytes due to RAG mutations: severe immunodeficiency with a spectrum of clinical presentations. Blood 2001; 97:81-88.

8. **Pasic S**, Jankovic I, Rosic R, Ognjanovic M. Pneumocystis carinii in haemophagocytic lymphohistiocytosis. Acta Paediatrica 2001; 90:1480-82.
9. Ziegner UHM, Kobayashi RH, Cunningham-Rundles C, Espanol T, Fasth A, Huttenlocher A, Krogstad A, Marthinsen L, Notarangelo LD, **Pasic S**, Rieger CHL, Rudge P, Sankar R, Shigeoka AO, Stiehm ER, Sullivan K, Webster AB, Ochs HD. Progressive neurodegeneration in patients with primary immunodeficiency disease on IVIG treatment. Clin Immunol 2001; 102:19-24.
10. **Pasic S**, Pavlovic M, Vojvodic D, **Abinun M**. Pityriasis Lichenoides in a Girl with the Granulomatous Form of Common Variable Immunodeficiency. Pediatr Dermatol 2002;19(1):56-59.
11. **Pasic S**, Minic A, Minic P, Veljkovic D, Lilic D, Slavkovic B, Pejnovic N, **Abinun M**. Long-Term Follow-Up and Prognosis of Chronic granulomatous disease in Yugoslavia: Is There a Role for Early Bone Marrow Transplantation ?. J Clin Immunol 2003; 23: 55-61.
12. Pirovano S, Mazzolari E, **Pasic S**, Albertini A, Notarangelo LD, Imberti L. Impaired thymic output and restricted T-cell repertoire in two infants with immunodeficiency and early-onset generalized dermatitis. Immunol Lett. 2003; 86: 93-7.
13. Marjanovic B, Stojanov Lj, Zamurovic D, **Pasic S**, Kravljanac R, Djordjevic M. Fulminant subacute sclerosing panencephalitis: two cases with atypical presentation. Pediatr Neurol 2003; 29: 63-65.
14. **Pasic S**, Micic D, Kuzmanovic M. Epstein-Barr virus-associated haemophagocytic lymphohistiocytosis in Wiskott Aldrich syndrome.Acta Paediatr 2003; 92: 859-861.
15. **Pasic S**, Minic P, Dzudovic S, Minic A, Slavkovic B. Idiopathic CD4 lymphopenia and juvenile laryngeal papillomatosis. Pediatr Pulm 2005; 39:281-3.
16. Mazzolari E, Moshous D, Forino C, De Martiis D, Offer C, Lanfranchi A, Giliani S, Imberti L, **Pasic S**, Ugazio AG, Porta F, Notarangelo LD. Hematopoietic stem cell transplantation in Omenn syndrome: a single-center experience. Bone Marrow Transplant 2005; 36:107-114.
17. **Pasic S**, Vujic D, Djuricic S, Jevtic D, Grujic B. Burkitt lymphoma-induced ileocolic intussusception in Wiskott Aldrich syndrome. J Pediatr Hematol Oncol 2006; 28:48-49.
18. **Pasic S**, Minic A, Djuric P, Micic D, Kuzmanovic M, Sarjanovic Lj, Markovic M. Fever of unknown origin in 185 paediatric patients: a single-center experience. Acta Paediatr 2006; 95:463-6.
19. Minegishi Y, Saito M, Morio T, Watanabe K, Agematsu K, Tsuchiya S, Takada H, Hara T, Kawamura N, Ariga T, Kaneko H, Kondo N, Tsuge I, Yachie A, Sakiyama Y, Iwata T, Bessho F, Ohishi T, Joh K, Imai K, Kogawa K, Shinohara M, Fujieda M, Wakiguchi H, **Pasic S**, **Abinun M**, et al.Human tyrosine kinase 2 deficiency reveals its requisite roles in multiple cytokine signals involved in innate and acquired immunity.Immunity. 2006; 25:745-55.
20. Trottestam H, Beutel K, Meeths M, Carlsen N, Heilmann C, **Pasić S**, Webb D, Hasle H, Henter JI. Treatment of the X-linked lymphoproliferative, Griscelli and Chédiak-Higashi syndromes by HLH directed therapy. Pediatr Blood Cancer. 2009; 52:268-72.
21. Jovanovic A, Minic P, Scekic-Guc M, Djuricic S, Cirkovic S, Weemaes C, **Pasic S**. Successful treatment of Hodgkin lymphoma in Nijmegen breakage syndrome. J Pediatr Hematol Oncol. 2009; 31:49-52
22. **Pasic S**, Djuricic S, Ristic G, Slavkovic B. Recombinase-activating gene 1 immunodeficiency: different immunological phenotypes in three siblings. Acta Paediatr. 2009; 98:1062-4.
23. Tóth B, Volokha A, Mihas A, Pac M, Bernatowska E, Kondratenko I, Polyakov A, Erdos M, **Pasic S**, et al. Genetic and demographic features of X-linked agammaglobulinemia in Eastern and Central Europe: a cohort study. Mol Immunol. 2009; 46:2140-6.
24. Walter JE, Rucci F, Patrizi L, Recher M, Regenass S, Paganini T, Keszei M, Pessach I, Lang PA, Poliani PL, Giliani S, Al-Herz W, Cowan MJ, Puck JM, Bleesing J, Niehues T, Schuetz C, Malech H, DeRavin SS, Facchetti F, Gennery AR, Andersson E, Kamani NR, Sekiguchi J, Alenezi HM, Chinen J, Dbaibo G, ElGhazali G, Fontana A, **Pasic S**, Detre C, Terhorst C, Alt FW, Notarangelo LD. Expansion of immunoglobulin-secreting cells and defects in B cell tolerance in Rag-dependent immunodeficiency. J Exp Med. 2010; 207:1541-54.
25. Booth C, Gilmour KC, Veys P, Gennery AR, Slatter MA, Chapel H, Heath PT, Steward CG, Smith O, O'Meara A, Kerrigan H, Mahlaoui N, Cavazzana-Calvo M, Fischer A, Moshous D, Blanche S, Pachlopnick-Schmid J, Latour S, de Saint-Basile G, Albert M, Notheis G, Rieber N, Strahm B, Ritterbusch H, Lankester A, Hartwig NG, Meyts I, Plebani A, Soresina A, Finocchi A, Pignata C, Cirillo E, Bonanomi S, Peters C, Kalwak K, **Pasic S**, et al. X-linked lymphoproliferative disease due to

SAP/SH2D1A deficiency: a multicenter study on the manifestations, management and outcome of the disease. Blood 2011; 117:53-62.
26. Gulácsy V, Freiberger T, Shcherbina A, Pac M, Chernyshova L, Avcin T, Kondratenko I, Kostyuchenko L, Prokofjeva T, **Pasic S**, et al. J Project Study Group. Genetic characteristics of eighty-seven patients with the Wiskott-Aldrich syndrome. Mol Immunol 2011; 48:788-92.

Letters, short notes

1. **Pasic S**. Immunodeficiency with Hyper-immunoglobulin M in an infant who had cryptosporidiosis assocaiated with interferon-γ deficiency. Clin Infect Dis 1996; 23:1336.
2. **Pasic S**. Local bacillus Calmette-Guerin infection in hyperimmunoglobulin E syndrome. Acta Pediatr 2002; 91:1271-2.
3. **Pasic S**. Aplastic anemia in Nijmegen breakage syndrome. J Pediatr 2002;141:742.
4. **Abinun M,** Lilic D, Gagic N, **Pasic S**. Long term follow-up of children with primary hypogammaglobulinemia in Yugoslavia. Clin Immunol 2003; 107: 202-203.
5. **Pasic S,** Savic Dj, Milovic I, Vasiljevic Z, Djuricic S. Propionibacterium propionicus infection in chronic granulomatous disease. Clin Infect Dis 2004; 38:459.
6. **Pasic S.** Autoimmune cytopenia in common variable immunodeficiency. J Pediatr 2004; 144: 689.
7. Kuzmanovic M, Rasovic N, Micic D, Bunjevacki G, Cupic V, **Pasic S**. Epstein-Barr virus associated hemophagocytic lymphohystiocytosis during maintenance treatment of acute lymphoblastic leukemia. Pediatr Blood Cancer 2006; 46:832.
8. **Pasic S**. Intravenous immunoglobulin in toxic epidermal necrolysis. Int J Dermatol. 2006; 45:1117-8.
9. Minegishi Y, Saito M, Tshuchiya S, Tsuge I, Takada H, Hara T, Kawamura N, Ariga T, **Pasic S**, Stojkovic O, Metin A, Karasuyama H. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. Nature 2007; 448: 1058-62.