Abstract Book

1st Early Career Investigators' (ECI) Workshop

Basic and Clinical Research in Chronic Neutropenias

September 02-03, 2021, Genova, Italy

1st Early Career Investigator's (ECI) Workshop

cost
EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

GASLINI

EuNet INNOCHRON

Welcome,

It is our great pleasure to welcome you to Genova for the first Early Career Investigators' Meeting (ECI) within the framework of the COST Action 18233, EuNet-INNOCHRON, a European Consortium aimed to widespread knowledge and increment networking on chronic neutropenias.

This two-day event is specifically designed to offer young investigators the opportunity to show their work and discuss among peers their research programs on neutropenia and related disorders thus providing a unique opportunity to create new interactions and to further implement already ongoing collaborations.

In addition to a specific session dedicated to selected oral and poster presentations, the meeting will also provide several chances to discuss new ideas and already set research programs with the most eminent international experts in the fields who operate as senior members of the EuNet-INNOCHRON Consortium.

Overall, this appears as a great opportunity for young researchers to consolidate and further increase their knowledge and expertise and to create new working opportunities and social contacts in a scientifically top level, but still very friendly, atmosphere.

While the prestigious Villa Canali Gaslini in Genova, the venue of the G. Gaslini Foundation, a historical dwelling built in 1924-25 by the famous Florentine architect Gino Coppedè, warmly welcomes all participants, we wish a very pleasant and fruitful attendance to this first edition of the ECI meeting.

The local organizers:

Carlo Dufour, MD

Vice Chair of the COST Action 18233, EuNet-INNOCHRON

Chair of SWG on Granulocytes and Constitutional Marrow Failure of European Hematology Association (EHA)

Francesca Fioredda, MD

MC member of the COST Action 18233, EuNet-INNOCHRON

The Coordinators:

Helen A. Papadaki, MD, PhD

Chair of the COST Action 18233, EuNet-INNOCHRON

Irene Mavroudi, PhD

MC member & Grant Holder Manager of the COST Action 18233, EuNet-INNOCHRON

General Information

Meeting Venue
FONDAZIONE GEROLAMO GASLINI
Corso Italia 26 – 16145 Genova
https://www.fondazionegaslini.org/home/en/

Map to Fondazione Gerolamo Gaslini

Genova Practical Information

Discover Genova

Genova is the capital of the Liguria region. It is the most important harbour in Italy and one of the main harbours in the Mediterranean Sea.

Find more about the City, Transportation and other practical Information here: www.visitgenoa.it/en

Program

Day 1 - September 2
WORKSHOP Part 1 (08:30 - 18:30)

8:30 - 9:00 REGISTRATIONS
9.00 - 10.30 INTRODUCTORY SESSION
Chairs: Carlo Dufour & Helen Papadaki

– Welcome to all participants by EuNet-INNOCHRON Chair (Helen Papadaki)
– Welcome by Local Organisers, practical Information and overview of the meeting-Institution virtual tour (Carlo Dufour)
– Presentation of the Young EuNet-INNOCHRON Group: Aims and Perspectives (Maksim Klimiankou)
– Speed dating round of ECIs: Who are they? What is the focus of their work? In which neutropenia type? Which are the diseases and methods they are interested in learning/establishing?

10:30 - 11.00 Coffee break

11:00 - 17:00 GROUP SESSIONS - PART I
Separate pre-defined groups of ECIs will try to work on preselected questions/queries on Chronic Neutropenias under the supervision of facilitators/tutors. Their findings and proposals will be presented and discussed in the second day of the Meeting.

11:00 - 12:30 Group Session 1:
Question/Query: Immune disturbances in patients with CNPs - Covid-19 infection and neutropenia
Give in brief the main literature. List the most used and useful assays. Discuss potential research projects.
Facilitator/Tutor: Valentino Bezzerri

12:30 - 14:00 Group Session 2:
Question/Query: Panels for molecular genetic diagnostics of CNP – prevention and early diagnosis of MDS/AML transformation (congenital and acquired)
Discuss their usage. Provide literature. Propose algorithms.
Facilitator/Tutor: Maksim Klimiankou

14:00 - 15.00 Lunch break

15:00 - 16:30 GROUP SESSIONS - PART II

15:00- 16:30 Group Session 3:
Question/Query: iPSC-based models to study the pathogenesis of CNP comparison with other models
Discuss their usage. Where is the field now? What are the current feasible applications.
Facilitator/Tutor: Benjamin Dannenmann

16:30 - 17:00 Coffee break

17:00 - 18:30 POSTER WALKS
Facilitator: Orna Steinberg-Shemer, Marije Bartels

End of Day 1

Day 2 - September 3
WORKSHOP Part 2 (09:00 - 18:30)

9:00 - 10:30 - "MEET THE EXPERT" SESSION PART I (Piero Farruggia)
Groups chaired by experts. Registration in advance.

9:00 - 9:45 Group 1. Congenital Neutropenia: Molecular Diagnosis - from the bedside to the bench and back
Experts: Alan Warren, Marco Cipolli

9:45-10:30 Group 2. Acquired Neutropenias in adults: Diagnosis and monitoring
Experts: Jan Palmblad, Helen Papadaki

Coffee break: 10:30-11:00

11:00 - 13:15 - "MEET THE EXPERT" SESSION PART II (Piero Farruggia)

11:00 - 11:45 Group 3. How to make a diagnosis of autoimmune neutropenia
Experts: Francesca Fioredda, Petter Höglund

11:45- 12:30 Group 4. Basic and Translational Research within EuNet-INNOCHRON - opportunities for STSMs
Experts: Julia Skokowa, Ivo Touw

12:30 - 13:15 Group 5. The impact of Registries in Optimizing the Care of Patients with CNP
Experts: David Dale and panel discussion by Jean Donadieu, Francesca Fioredda, Cornelia Zeidler

13:15 - 14:30 - Lunch Break

14:30 - 16:00 Presentations by the ECIs on the topics already discussed and prepared in ECI Workshop part I (Day 1 of the meeting)
Chair: Karl Welte

14:30 - 15:00 Group 1: Immune disturbances in patients with CNPs - Covid-19 infection and neutropenia
Speaker(s): (Valentino Bezzerri and young investigators under the supervision of the tutors of day 1)

15:00 - 15:30 Group 2: Panels for molecular genetic diagnostics of CNP - prevention and early diagnosis of MDS/AML transformation (congenital and acquired)

Speaker(s): (Maksim Klimiankou and young investigators under the supervision of the tutors of day 1)

15:30 - 16:00 Group 3: iPSC-based models to study the pathogenesis of CNP comparison with other models
Speaker(s): (Benjamin Dannenmann and young investigators under the supervision of the tutors ofday 1)

16:00 - 16:30 Coffee Break

16:30 - 17:30 ORAL PRESENTATIONS OF 4 SELECTED ABSTARCTS
Chair: Marije Bartels, Orna Steinberg-Shemer

17:30 - 18:30 SYMPOSIUM IN APPLYING FOR A GRANT OR MAKING CAREER PLANS
Chairs: David Dale, Carlo Dufour
Panel: Julia Skokowa, Helen Papadaki, Karl Welte

End of Day 2 and end of the meeting

ABSTRACTS for
ORAL PRESENTATION

“Late onset and long-lasting neutropenia: epiphenomenon of immune dysregulation”

Andrea Beccaria, Cecilia Contratto, Eleonora Rotondo, Paola Terranova, Marina Lanciotti, Alice Grossi, Isabella Ceccherini, Giovanni Del Borrello, Maurizio Miano, Carlo Dufour, Francesca Fioredda

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Background: Long lasting and late onset autoimmune and idiopathic neutropenia (AN and IN) are probably different entities if compared to primary and secondary forms.

Aims: To analyze a cohort of patients affected with AN/IN rising > 3 years with duration >12 months or rising <3 years with persistence >36 months.

Methods: Collection of clinical, immunological and genetic data (NGS panel of 160 immunodeficiency/disimmunity genes) of eligible patients within the Italian Neutropenia Registry.

Results: From 2005 to 2020 were collected data from 46 patients (24F, 24 AN, 22 IN) with median age at onset of 11.2 years (IQR13.2-16.7) and a median follow-up of 4.3 years (IQR 3.2-6.8). Neither autoimmunity nor cytopenias were present at onset. Autoimmunity occurred with a cumulative incidence of 11.8% at 5 years after onset (CI 95%4.3-30). In the whole cohort, the median values of leucocytes and lymphocytes at the beginning was significantly higher than those seen at last control ($p < 0.001$). In particular, B and NK lymphocytes had lower values compared to the normal ranges, as did B switched memory CD27+/IgD-/IgM-(in 94%).Conversely, B lymphocytes CD27+/IgD+/IgM+ (62%) and Tγδ (70%) increased according to a pattern comparable to that described in chronic idiopathic neutropenia (CIN). The genetic study showed 5/32 (16%) cases pathogenic variants: 2TACI, 1TINF2, 1CARD11 of immunodeficit/dysregulation and in 9/32 (28%) cases (4 CASP10, 1DDX41/SCOCS1, 1TSR2/DCLRE1c,1PIKSD,1 TERT/TACI) variants of uncertain significance.

Conclusions: Late-onset and long lasting AN and IN in infancy would be an anticipation of “more complex“ disorders which may develop into secondary AN (onset of autoimmunity) or CIN. Some genetic variants could explain the immunodysregulation at least in a subgroup. Definitive conclusions will be drawn by applying more “accurate” methods (Whole Exome Sequencing and Whole Genome Sequencing) on largest groups of subjects.

“Zebrafish models for severe congenital neutropenia”

Larissa Doll

University Hospital Tübingen, Ottfried-Müller, Tübingen, Germany

Background/Aim: Severe congenital neutropenia (CN) is a bone marrow failure syndrome characterized by a neutrophil deficiency. CN patients are prone to develop life-threatening infections from birth on and in long term they are prone to develop MDS or AML. This disease can be caused by different germline mutations, like HCLS1 associated protein X-1 (HAX1) or Jagunal homolog 1 (JAGN1), among many others. The underlying mechanism is still poorly understood.

Our current knowledge of *HAX1*- and *JAGN1*-associated neutropenia is predominantly derived from clinical observations and *in vitro* studies. Thus far, no reliable *in vivo* model has been established. To overcome the limitation and further analyze the function of HAX1 and JAGN1 in granulopoiesis and neutropenia, we have established CN zebrafish models.

Methods/Results: To interfere with the function of the proteins we used two different approaches. First, we injected an antisense morpholino which efficiently blocked the translation. Second, we used CRISPR/Cas9 to introduce mutations into the genes. Later, to analyze the effects on the different hematopoietic cell types (neutrophils, macrophages, erythrocytes) in the different morphants/mutants we quantified the cell number using whole-mount in situ hybridization against different marker genes.

After interfering with the function of jagn1b or hax1, either using morpholino or CRISPR/Cas9, the numbers of neutrophils were significantly reduced without affecting the early myelopoiesis, monopoiesis or erythropoiesis. Further analysis also revealed that apoptosis was enhanced in the hax1 and jagn1b morphants, however, this effect was not restricted to the hematopoietic tissues.

Conclusion: Overall, we have established two novel *in vivo* models for studying the role of HAX1 and JAGN1 in granulopoiesis, which might be useful to understand better the underlying mechanism of the disease and therefore lead to the discovery of new targets for the development of therapeutic strategies for CN.

"Lab-on-chip for studying chemotaxis in monocytes and neutrophils isolated from patients with Shwachman-Diamond syndrome"

Nora Selicato, Eleonora De Vitis, Francesca Gervaso, Alessandro Polini, Valentino Bezzerri and Marco Cipolli

Azienda Ospedaliera Universitaria Integrata, Verona, Italy

Background: Shwachman-Diamond syndrome (SDS) is a rare inherited multisystemic syndrome characterized by bone marrow failure, exocrine pancreatic insufficiency, associated with juvenile myelodysplastic syndrome and high risk of leukemic transformation. SDS is mainly caused by mutations in the Shwachman-Bodian-Diamond Syndrome (*SBDS*) gene encoding a protein involved in ribosomal biogenesis. Interestingly, three other genes involved in ribosome biogenesis have recently been associated with SDS-like phenotype, strengthening the postulate that SDS is a ribosomopathy. No pharmacological therapy for SDS has been developed so far. Promising new therapies aiming to correct the genetic defect have been supposed. Since most patients with SDS exhibit nonsense mutations in the *SBDS* gene, recently the drug repositioning of the small nonsense suppressor molecule, namely ataluren [PTC124; 3-(5-(2-fluorophenyl)-(1,2,4)oxadiazol- 3- yl)-benzoic acid] has been proposed. Ataluren has been already approved by the European Medicines Agency for the treatment of Duchenne muscular dystrophy. In previous studies, ataluren significantly restored SBDSprotein expression in bone marrow progenitor cells from patients with SDS, promoting myeloid differentiation. One of the pathogenetic hallmark of the SDS patients is the impaired neutrophil and monocyte chemotaxis defect, that was previously studied with traditional cell migration assays, which cannotprovide the temporal and spatial control of the chemical gradients, neither study the single cell behaviour.

Aim: Lab-on-chips are a new research platform for studying chemotaxis and chemokinesis able to overcome the limitations of traditional chemotaxis assays due to the advantage of visualization, precise control of the chemical gradient and small consumption of reagents. In this study, we developed a lab-on-chip platform and evaluated ataluren potential in restoring chemotaxis.

Methods: We fabricated a flow-free microfluidic device with three different perfusable compartments with distinct inlets and outlets, interconnected through a series of microchannels that can be used to control cell migration and cell differentiation on chip. The platform was fabricated by SU-8-based multi-level photolithography and PDMS replica molding. Gradient profiles were previously evaluated to ensure an adequate time to perform the chemotaxis experiments and a preliminary cell line was used to set up the device for the purpose of this study. We tested ataluren potential in restoring the chemotaxis, treating neutrophils (PMN) differentiated from bone marrow mononuclear cells (BM-MNCs) obtained from patients carrying the c.183-184TA>CT nonsense mutation. At the seeding time, ataluren was added at different concentrations in the culturing medium supplemented with 20 ng/ml G-CSF. Neutrophil maturation was determined after 24h by flow cytometry. Then, the chemotaxis assay was performed, seeding $1.5x10^4$ cells in the lowest compartment and the cell migration was evaluated in the presence or in the absence of chemotacticstimuli (IL-8 and fMLP) injected in the upper compartment of the device. Brightfield time-lapse images of cell migration were captured for 1 hour and the cell trajectories analysed using Manual Tracking ImageJ software. Then, the data were further analysed to obtain thetrajectory plots and the main output parameters for chemotaxis.

Results/Discussion: Our results indicated that our chemotaxis-on-chip device is useful to study single-cell chemotaxis. Furthermore, our preliminary results suggest that ataluren treatment could partially improve the chemotaxis capabilities of SDS neutrophils.

"Follow-up Next Generation Sequencing Analyses on clonal CIN patients: novel insights on the natural history of this rare disease"

Grigorios Tsaknakis, Stavros Papadakis, Peggy Kanellou, Irene Fragiadaki, Irene Mavroudi, Charalampos Pontikoglou, Anna Galli, Luca Malcovati, Helen Papadaki

School of Medicine, University of Crete, Heraklion, Crete, Greece

Background: We have previously performed NGS analysis of genes that are recurrently mutated in myeloid malignancies, in a cohort of patients with the diagnosis of chronic idiopathic neutropenia (CIN) according to previously reported criteria that largely overlap with the ICUS-N proposed criteria. We have estimated for the first time the frequency of clonal hematopoiesis in patients with CIN/ICUS-N (11.54%) and found that clonal CIN patients have a significantly higher risk of developing a myeloid neoplasm than those with no evidence of clonality (non-clonal). However more longitudinal follow-up NGS studies are required for the tracking of clonal evolution and delineation of CIN natural history.
Aims: To conduct longitudinal follow-up NGS analyses in order to assess clonal evolution and clinical significance of detected clonal aberrations in CIN clinical outcome.

Methods: Genomic DNA was extracted from patients' BM or PB samples, sequencing libraries were prepared and subjected to targeted next generation sequencing (NGS) on an Ion S5 Prime Sequencer (Thermo Fisher Scientific) using a panel of 38 genes recurrently mutated in myeloid malignancies.

Results: Follow-up analysis by NGS was performed in 17 clonal CIN patients. The median time between the first and subsequent analysis was 23 months (range 4-164 months). Ten of these patients carried the initial somatic mutations with only subtle changes in VAF and displayed absence of additional mutations and stable disease course. Two patients acquired a second mutation at follow-up. One of them still displayed stable disease course whereas the second eventually progressed to CMML. The analysis also revealed that one patient lost the initial detected mutation at follow-up. Two patients who progressed to MDS/MPN and AML respectively displayed a notable clonal expansion with additional mutations at the time of progression. Specifically, the patient who progressed to MDS/MPN acquired a mutation in *JAK2* and *ASXL1* while the patient who progressed to AML acquired the typical *NPM1*p.L287fs mutation. The patient who developed MDS with multilineage dysplasia, carrying three mutations in *DNMT3A* and *IDH1*, showed a moderate increase in the VAF of these mutations at follow-up.

Conclusions: In the majority of patients tested for clonal evolution over time, most single mutant clones appeared to be remarkably stable, with minimal VAF change, no acquisition of new molecular alterations and stable disease status. Two CIN patients who transformed to a myeloid malignancy displayed a clonal expansion as was reflected by the increase of VAF and the development of additional mutations whereas in the third patient only a modest VAF increase was identified. This ongoing study of sequential NGS analysis of CIN patients is anticipated to enrich further the knowledge on the natural history of this rare disease.

ABSTRACTS for
POSTER PRESENTATION

"Leucopenia with lymphocytosis in childhood and adolescence. Course and prognosis of referrals at a Pediatric Hematology department"

Emmanouil Athanasopoulos, Nikolaos Katzilakis, Iordanis Pelagiadis, Maria Stratigaki, Eftichia Stiakaki

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Introduction: Lymphocytosis refers to an increase in the number of lymphocytes in the blood. Absolute lymphocytosis is characterized by an increase in the lymphocyte count above the normal range; in children older than 12 years above the 4000/μL threshold and in younger children above 8000 /μL. Relative lymphocytosis refers to an increase in the lymphocyte proportion relative to the white blood cell count. Lymphocytosis constitutes a common finding in children and adolescents. In the majority of cases, an underlying cause cannot be identified, and the condition is self-resolving, while less often, especially when associated with leukopenia and neutropenia, further investigation is warranted.

The aim of our study is to investigate the epidemiological, clinical and laboratory characteristics of children and adolescents that have been evaluated in a tertiary hospital with lymphocytosis in a 5-year period.

Patients and Methods: A retrospective cohort study was performed and demographic, clinical and laboratory data were collected from children and adolescents that were referred to the outpatient services of the Paediatric Haematology – Oncology Department of the University Hospital of Crete due to lymphocytosis. Statistical analysis was conducted using Graph Pad Prism version 9.1.2 ©. statistical software. Mann-Whitney test was used to compare categorical variables while continuous variables were compared by Fisher's exact test. The odds ratios (OR) were calculated and a p value <0.05 was considered statistical significant.

Results: A total of 103 patients with lymphocytosis were included in the study; 47 females and 56 males. The age range at diagnosis was 6.06 to 17.35 years old (mean 10.87 years). In 83.65% of cases no underlying cause was identified. Causes of lymphocytosis included: hypothyroidism in 7.77% of cases, viral infections (0.97% Epstein – Barr virus, 0.97% Mumps, 0.97% Adenovirus) and antiepileptic treatment (1.94% Valproic Acid, 0.97% Levetiracetam). One case was attributed to autoimmune condition and one case to possible Myelodysplastic syndrome. In most of the cases (73.79%), the lymphocytosis resolved within 16.14 months from the time of diagnosis. In addition, 41.75% had recovered on the first visit. Patients with lymphocytosis due to an underlying cause versus those without cause differed significantly in terms of white blood cell count (p value 0.01), mean absolute neutrophil count, (p value 0.006) and the mean absolute lymphocyte count, (p value 0.01). Also, patients whose lymphocytosis resolved versus those whose lymphocytosis remained differed significant in terms of older age at diagnosis and presence of relative lymphocytosis (p value 0.04 and p 0.008 respectively).

Conclusions: In agreement with the existing literature, lymphocytosis in healthy children is often a self-resolving condition with good prognosis. The underlying causes are endocrine, viral infections, drugs and rarely autoimmune and hematological conditions. Low white blood cell count, low neutrophil at diagnosis are more commonly associated with an underlying cause while older age at diagnosis and the presence of relative lymphocytosis are associated with full recovery.

“Studying immune deregulation in Chronic Idiopathic Neutropenia (CIN): The immunoregulatory cell populations”

Nikoleta Bizymi, Nikoletta Aresti, Athina Damianaki, Maria Velegraki, KonstantinaZavitsanou, Anastasios Karasachinidis, Anthie Georgopoulou, Irene Mavroudi, Charalampos Pontikoglou, Helen A. Papadaki

Haemopoiesis Research Laboratory, School of Medicine, University of Crete, Heraklion, Crete, Greece

Introduction: Chronic Idiopathic Neutropenia (CIN) is characterized by prolonged and unexplained reduction in the number of peripheral blood (PB) neutrophils and Increased an inhibitory bone marrow (BM) micro-environment with over-activated oligoclonal T-cells and increased pro-inflammatory cytokines *(Bizymi et al, J Clin Immunol 2019)*. The aim of the present study is to explore the possible involvement also of the immunoregulatory populations, i.e. Myeloid-derived suppressor cells (MDSCs) and T regulatory cells (T-regs), in the pathophysiology of CIN. MDSCs are divided in HLA-DR$^{low/-}$/CD11b^{+}/CD33^{+}/CD15^{+} polymorphonuclear (PMN-MDSCs) and HLA-DR$^{low/-}$/CD11b^{+}/CD33^{+}/CD14^{+} monocytic (M- MDSCs) subsets *(Bizymi et al, HemaSphere 2019)*. Natural T-regs (nT-regs) express constitutively CD4, CD25 and FoxP3. MDSCs induce the production of T-regs. These immunoregulatory cell populations limit T cell responses, are important for immune homeostasis and are known to be implicated in inflammatory and malignant diseases.

Methods: 100 CIN patients and 49 age- and sex-matched healthy controls were studied. The patients had mean neutrophil counts 1095.67 ± 479.52 (median 1215, range 100-1700). M- MDSC, PMN-MDSCs and T-regs were quantitated by flow cytometry in the PB mononuclear cell (PBMC) fraction. The BMs of 24 CIN patients and 8 healthy controls from the study population were also analysed. Statistical analysis was performed for unpaired data with the Mann-Whitney test, while for paired data with the Wilcoxon test.

Results: We found that the population of PB M-MDSCs was statistically significant lower in CIN patients (1.45 % ± 1.82) compared to controls (3.68 % ± 3.12, $p < 0.0001$) whereas the proportion of Tregs was increased in the PBMC fraction of the patients (0.46 % ± 0.28) compared to the healthy controls (0.24% ± 0.17, p=0.0011). Paired analysis showed that the population of PMN-MDSCs was higher in the BM compared to the PB in both CIN patients (13.27 % ± 11.27 vs 1.14 % ± 1.64, respectively, $p = 0.005$) and healthy controls (19.49% ± 4.46% vs 9.92% ± 9.08%, respectively, $p = 0.0118$). The proportion of increase of PMN-MDSCs(in BMMC vs PBMC fraction) was significantly higher in patients (86.71 % ± 21.26%) comparedto controls (55.95 % ± 38.59%, $p = 0.0357$). This indicates impaired production of PMN-MDSCsin CIN patients compared to controls but a trend for accumulation of these cells in patients’ BM. Of note, the numbers of PB CD4^{+}CD25$^{high\text{-}high}$FoxP3^{+} T-regs correlated with the numbers of PB PMN-MDSCs in CIN patients (Spearman Correlation, $r = 0.3683$, $p = 0.0229$).

Conclusions: CIN patients display lower proportions of MDSCs and higher proportions of T- regs in the PB compared to normal individuals. Patient MDSCs seem to display normal capacityto induce T-regs. The production of MDSCs is impaired in CIN in a different manner than the production of T-regs, which is normal, and CIN T-regs are induced by several pathways besidesMDSCs. The low proportions of MDSCs may sustain the inflammatory process associated withCIN whereas the accumulation of PMN-MDSCs in the BM and the elevated levels of T-regs in the PB may represent a compensatory mechanism to suppress the inflammatory processes within patients’ BM microenvironment.

"NeutroApp: An electronic database for the registration of patients with Chronic Idiopathic Neutropenia with interconnection potential"

Erasmia Boutakoglou, Peggy Kanellou, Stavros Papadakis, Irene Mavroudi, George Chalkiadakis, Helen Papadaki

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Background: Chronic Idiopathic Neutropenia (CIN) in adults is a rare disorder of granulopoiesis characterized by the prolonged and unexplained reduction of peripheral blood (PB) neutrophil counts. The diagnosis of CIN is based on exclusion of neutropenia-associated entities and the pathophysiology implicates different underlying mechanism. The application of molecular techniques has surprisingly shown that among European patients initially characterized as CIN there are 4.5%-5% patients with *DARC/ACKR1* polymorphisms and Fy(a-b-) red blood cell phenotype. In addition, the wide application of next generation sequencing (NGS) of genes associated with myeloid malignancies has identified clonal haemopoiesis (CH) in approximately 11% of CIN patients conferring a high positive predictive value for MDS/AML evolution. Registration of CIN patients to databases and longitudinal follow-up is of particular importance for the better diagnosis and prognosis of the patients and clarification of CIN natural history.

Aims: Our goal is to set up a user-friendly electronic database to register the clinical and laboratory data of CIN patients, at diagnosis and follow up, as well as the disease outcome. An interconnection potential with existing international registries to enrich their content, should ideally characterize the application (app).

Methods: We have set up the "NeutroApp" electronic database and CIN patient registry consisting of a repository (Relational Data Base System) and web interfaces to registered users and administrators. It is hosted by the University of Crete's Data Center, thus fulfilling criteria of access control, safety of personal data (according to General Data Protection Regulation; GDPR), loss of data and computing response time. It provides graphical user interface (GUI) masks for patient's data, reports based on the data sets, and statistical graphs for users and administrators. Furthermore, it provides the means for integration and data exchange with similar registries using HL7 (transfer of clinical and administrative data protocol). At the time of diagnosis, we enter patient basic laboratory tests (i.e. cell blood counts, serum biochemistry, hormonal and immunoglobulin results, antibodies to viral and bacterial pathogens, immunophenotype of PB and bone marrow [BM], results from BM morphology, trephine biopsy, karyotype). We also enter data from advanced techniques such as anti-neutrophil antibodies following leucoagglutination and immunofluorescence, polymorphisms of *DARC/ACKR1*following real-time PCR, and NGS of a panel of myeloid genes, among others. Selective data are also entered during the follow-up of the patients every 3-12 months.

Results: So far, we have registered 204 adult patients initially diagnosed as CIN and further characterized as autoimmune, Fy(a-b-), familial with undefined genetic defect, clonal, non-clonal. We longitudinally monitor the registered patients with an intent to add more from different centers within EuNet-INNOCHRON; we also intent to investigated the potential to automatically enrich the Severe Chronic Neutropenia International Registry (SCNIR) with the severe CIN cases, through the "NeutroApp" interconnection capability. The registration of patients is anticipated to broaden the knowledge in the field of CIN and to promote collaborations within EuNet-INNOCHRON.

“Steps in diagnosis of chronic idiopathic Neutropenia”

Aleksandra Catić-Đorđević, Sanja Veličković,Miodrag Vučić, Filip Veličković

Faculty of Medicine, University of Nis, Nis, Serbia

Background: Neutropenia that persists more than 3 months and not associated with medications use, infectious disease, inflammation, autoimmune diseases and malignancies is defined as chronic idiopathic neutropenia (CIN). CIN is often asymptomatic condition, but required monitoring regarding possibilities of undesired health outcomes.

Aim: The aim was to show stratified diagnostic approach towards patient with longtime low neutrophils count across case report of one patient. Presentation of diagnostic procedure include timely patient recognition with adequate follow-up and formulation of action plan afterwards.

Methodology: A 40-year-old female patient comes to the Hematology Department at University Clinical Center for regular health condition and blood count control. Since she was 21, she has been following neutropenia, which occurred immediately after first giving birth. Regarding illuminate the type of neutropenia, we conducted graduated as similar diagnostic approach showed in European Network for Innovative Diagnosis and Treatment of Chronic Neutropenias (COST CA 18233) as possible at the moment.

Results: At first, patient undergone investigation aimed precise information related with patient’s characteristics, comorbidities, family history, and genetic predisposition to neutropenia. Therefore, we determined that the patient did not have a positive family history or comorbidities, excluding anemia, which also persisted in this patient since first child birth. As part of this step biochemical control showed: white blood count 2.2x10^9/L, neutrophils1.2 x10^9/L, eosinophils 0.1 x10^9/L, lymphocytes0.8 x10^9/L, monocytes0.1 x10^9/L, and basophils 0 x10^9/L, respectively. Red blood count, hemoglobin, hematocrit, number of platelets were in physiological range as well as level of folate, vitamin B12, hTSH, albumin and routine biochemical parameters, except iron which was low. The next step was immune analysis performed to find the immune background of neutropenia. Results were within the reference values: IgG 9.03 g/L, IgA 0.95 g/L, IgM 1.97 g/L, anti dsDNK6.2, ANA 0.1. Direct and indirect COOMBS tests were negative too. Next step performed in order to exclude viral infections as a cause of neutropenia. Serological tests regarding particular antibodies- HbS, HCV, CMV, EBV, Parvovirus, and HIV were negative. The next step represented the examination of a bone marrow of patient aimed to excluding any malignancy or haematological disorder which may be represented as peripheral blood neutropenia in patient. The bone marrow activity represented the next action, but not performed because of lack of technical condition in our institution. In addition, dietary habits of patient showed lack of proteins, particularly red meat, and higher than recommended carbohydrates intake. Nevertheless, BMI was 25 kg/m^2. All results lead to diagnosis of chronic neutropenia and future follow-up plan for this patient.

Conclusion: Particular steps in diagnostic procedure include timely patient recognition with appropriate follow-up and formulation of an action plan afterwards. For that reason, it is very important and useful to have a register with all data recorded in timely manner, which may help clinicians in routine monitoring and case of changes in patient’s health.

“Genetic diagnosis of inherited cytopenias associated with predisposition to leukemia in children and young adults”

Oded Gilad

Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel

Introduction: Prolonged cytopenia is a non-specific sign with a wide differential diagnosis including acquired and inherited disorders. Among inherited disorders, predisposition to leukemia has recently emerged as an important clinical entity. The overlap in clinical and laboratory presentation of those syndromes is high, while timely and accurate molecular diagnosis is essential to ensure appropriate medical management, including adequate monitoring and possibly stem cell transplantation prior to development of leukemia.

Aim: To evaluate the genetic diagnosis rate of patients with persistent cytopenia using NGS panel.

Methods: Children with prolonged cytopenias were referred to the central Molecular Hematology Laboratory of Schneider Children's Medical Center of Israel. In few patients with suggestive clinical picture and presence a known major commonly mutate gene the initial molecular diagnosis was based on Sanger sequencing. However, the majority of patients were diagnosed using custom-made targeted NGS panel covering 229 genes known to be mutated in IBMF, MDS predisposing syndromes and inherited thrombocytopenia (IT). Variants were classified according to the American College of Medical Genetics guidelines. Only pathogenic and likely pathogenic (P/LP) sequence changes were reported.

Results: Between 1.2016-12.2019, 173 children with persistent cytopenia underwent genetic evaluation. The median age at clinical presentation was 1 years (range 0.1 -37 years) and the median age at referral was 8 years (range 0.5-41 years). On average, patients were referred 5.7 years following their first clinical presentation. Thirteen patients were diagnosed initially by Sanger sequencing while 160 patients underwent NGS panel diagnosis. P/LP variants were identified in 56 patients (32.4%). Nine of those had IT affecting platelet production and function with no predisposition to leukemia, while 47 (27.2%) had leukemia predisposition. Sixty-three percent of patients with leukemia predisposition had IBMF, 19% had MDS, 14.2% had IT. Using NGS panel only one patient (1/30, 3.3%) with persistent neutropenia had a positive genetic diagnosis (homozygous for *JAGN1*mutation). By WES one additional patient was found to carry a novel *SRP54* mutation causing SCN. Following in corporation of this gene into our NGS panel we identified another patient with *SRP54* mutation. Additional patient with neutropenia and negative NGS-panel result was found by WES to be homozygous for *MUNC13* mutation. Five patients with no recurrent infections were further diagnosed as having benign neutropenia (*ACKR1/DARC* null polymorphism). All in all, by additional studies we were able to diagnose 30% of patients referred with persistent neutropenia.

Summary: About a third (32.4%) of 160 patients referred with persistent cytopenia, had an underlying inherited cause, with the majority (27.3%) having germline predisposition to leukemia. Using this precision medicine approach to identify children with cytopenia may offer a positive impact on prognosis and disease outcome.

"Functional impairment of neutrophils in chronic lymphocytic leukemia(CLL): A literature review and a rare case of pneumonia and bacteremia by*Listeria monocytogenes* complicating CLL"

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Background and Aims: Functional impairment of neutrophils in chronic lymphocytic leukemia (CLL) accounts for an increased susceptibility to bacterial infections, including uncommon infections from intracellular pathogens. We aim to summarize the negative effectson neutrophil count and function in CLL and their correlation with an increased risk for common and uncommon infections, as well as to present a rare case of a patient with CLL complicated by lethal pneumonia and bacteremia caused by the intracellular pathogen *Listeria monocytogenes*.

Literature Review: CLL is the most common type of leukemia in adults, and is characterizedby the abnormal expansion of malignant lymphocyte clones with devastating effects onhematopoiesis, including the production of neutrophils. In addition to the negative effects on neutrophil count, circulating neutrophils in CLL were previously found to be functionally impaired, possessing restricted bactericidal activity (Kontoyiannis et al, 2013), probably due to myeloperoxidase deficiency and impaired chemotaxis (Itala et al, 1996). Apart from their well-known central role in the defense against infections from extracellular pathogens, neutrophils were recently shown to have an essential contribution in infections from the intracellular pathogen *Listeria monocytogenes* by means of phagocytosis and production of bactericidal factors as part of novel bacterial-sensing pathways (Witter et al, 2016). In literature, only two cases of *Listeria monocytogenes* infection in CLL patients are reported: one with empyema and bacteremia (Myers & Rodgers, 1980) and one with meningoencephalitis (Bajko et al, 2013).

Case Presentation: In our center, an 83-year-old female patient with CLL diagnosed 10 yearsago (Binet stage C / Rai stage IV) presented with dyspnea, fever, and decreased level of consciousness. Both profile chest roentgenography and transthoracic ultrasonography revealedinfiltrates in the left lower lung field with a small ipsilateral pleural effusion, whereas the Gram-positive intracellular pathogen *Listeria monocytogenes* was isolated in blood cultures, but not in sputum cultures. Initially, the patient was treated empirically with piperacillin/tazobactam and levofloxacin, which was afterwards switched to ampicillin and gentamycin in accordancewith the antibiotic sensitivities of the pathogen. During her hospitalization, the patient was further complicated with septic shock which responded well to intravenous fluids and vasoconstrictors. She was unable to effectively increase the number of neutrophils, whereas the lymphocyte count reached more than 200,000 cells/μL, the platelet count was dropped to 50,000 cells/μL, and hemoglobin to less than 8 g/dL; thus, the patient was transfused with oneunit of packed red blood cells. The patient was also complicated with acute pulmonary edema,which was treated with diuretic therapy. After completing two weeks of antibiotic therapy, thepatient was discharged with long term oxygen therapy and per os diuretics. She eventually diedwithin two weeks, most probably due to recurrent acute pulmonary edema resulting from lackof compliance with the diuretic therapy.

Conclusion: Neutrophil functional impairment is as crucial as neutrophil count for the immuneresponse against bacterial infections in CLL. Patients failing to respond by increasing functional circulating neutrophils face high morbidity and mortality rates even from infectionscaused by otherwise rare pathogens.

“Can leukocyte proteases inhibitors be considered as new therapeutic targets for severe congenital neutropenia?”

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Background: SLPI (Secretory Leukocyte Protease Inhibitor) is a protein, present abundantly in mucous membranes andskin [1]. As shown by numerous studies, SLPI plays an important anti-pathogenic and anti-proteolytic role [2)]. Along with serpin-b1, SLPI is considered the main natural inhibitor of neutrophil elastase. Importantly, elastase mutations are the main cause of hereditary neutropenia, a group of diseases in which the number of neutrophils in the blood is significantly reduced. As shown by the latest research, diminished levels of SLPI are observed in myeloid cells and plasma of patients with severe congenital neutropenia [3]. Moreover, downregulation of SLPI in hematopoietic progenitors results in markedly reduced *in vitro* myeloid differentiation.

Methods/Results: Our results indicate, that among circulating leukocytes of healthy donors, SLPI is present only in granulocyte fraction, further suggesting that this protein is important for neutrophil function. Given its potential engagement in neutrophil precursor function, we asked, whether an increased amount of SLPI protein could help myeloid progenitors differentiate into mature neutrophils? To answer this question, we decided to use an *in vitro* model in which iPSCs (induced pluripotent stem cells) would be differentiated into granulocytes in cell culture. As a starting point, we tested several protocols for harvesting granulocytes from iPSC culture. The results obtained so far show, that the *in vitro* differentiation of iPSC cells allows us to obtain a heterogeneous population of granulocytes. Most of the analyzed cellsexpress surface markers of the myeloid lineage (CD33 / CD15 / CD11b). A smaller proportion of the cells have the markers of mature neutrophils, i.e. CD16 (20-30%) and CD10 (less than 10%). However, as shown by flow cytometry analysis and colorimetric tests of proteolytic activity, these cells have both active elastase and express SLPI protein. Through the use of the CRISPR-Cas9 approach, our goal is next to introduce most common mutations in the elastase gene in iPSC lines. Later, these lines will be differentiated into granulocytes under conditions of SLPI overexpression or exogenous administration. We hypothesized that an increased amount of SLPI protein may contribute to the improvement of the differentiation of myeloid progenitors with mutations in the elastase gene, thus increasing the number of neutrophils in the blood.

Discussion: Potential results of our research can help to discover new therapeutic approaches for severe congenital neutropenia.

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