Multidisciplinary approach in the diagnosis and management of Philadelphia chromosome-negative myeloproliferative neoplasms

Summary of the Ph.D. Thesis

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List of publications related to the thesis

I. Éva Pósfai, Imelda Marton, Zita Borbényi, Attila Nemes: Myocardial infarction as a thrombotic complication of essential thrombocythaemia and polycythaemia vera. The Anatolian Journal of Cardiology. 2016 IF 0.927 – accepted for publication – Co-authors Éva Pósfai and Imelda Marton contributed equally to this work and are equal in status.


V. Attila Nemes, Imelda Marton, Péter Domsik, Anita Kalapos, Éva Pósfai, Szabolcs Modok, Zita Borbényi, Tamás Forster Characterization of left atrial dysfunction in hypereosinophilic syndrome - Insights from the Motion analysis of the heart and great vessels by three-dimensional speckle tracking echocardiography in pathological cases (MAGYAR-Path) Study. Revista Portuguesa de Cardiologia. 2016– IF 0.454 accepted for publication –


1. INTRODUCTION

Myeloproliferative diseases (MPDs) or neoplasms (MPNs) represent a heterogeneous group of clonal haematopoietic stem cell (HSC) disorders. The so-called “classic” MPDs – now referred to as “classic” MPNs – include chronic myeloid leukaemia (CML), polycythaemia vera (PV), essential thrombocythaemia (ET), and primary myelofibrosis (PMF). The Polycythaemia Vera Study Group (PVSG) and the World Health Organization (WHO) classifications distinguish the Philadelphia chromosome (Ph)-positive CML from the Ph-negative entities ET, PV, and PMF.

The 2008 WHO classification of myeloid neoplasms provides a novel, morphology-, cytogenetics-, and molecular diagnostics-based nomenclature and classification of MPDs. In this revised classification, the expression “myeloproliferative disorder” has been replaced by “myeloproliferative neoplasm”. Eight clinicopathological entities fall therefore currently under the category of MPNs: BCR-ABL1-positive CML and seven BCR-ABL1-negative conditions including chronic neutrophilic leukaemia (CNL), PV, PMF, ET, CEL-NOS, mastocytosis, and MPNs – unclassifiable. The term “Ph-negative MPNs” is still widely used as a synonym for classic BCR-ABL1-negative MPNs (PV, ET, PMF) and, in the broader sense, also for other BCR-ABL1-negative myeloproliferative conditions such as certain rare entities like hypereosinophilic syndrome (HES) or systemic mastocytosis (SM).

Polycythaemia vera (PV) is the most common entity among all Philadelphia chromosome (Ph)-negative myeloproliferative disorders (MPDs) which, due to its vascular complications, represents an interdisciplinary significance. PV is characterized by the trilineage clonal proliferation of stem-cell derived haematopoietic progenitors resulting in the expansion of the erythrocyte mass. In addition to blood hyperviscosity, the increased red blood cell (RBC) mass results in a higher risk for thrombosis, poor quality of life, and a shorter life expectancy. PV warrants particular attention for the higher risk of cardiovascular (CV) and cerebrovascular events as leading determinants of morbidity and mortality. Predominant are arterial thrombotic events, in particular large vessel arterial events including cerebrovascular events, myocardial infarction, and peripheral arterial occlusion. From clinical and therapeutical aspects, the role and significance of additional risk factors in the development of PV-associated thrombotic events is of major importance – over the last few years, this topic has been actively investigated.

Systemic mastocytosis (SM), an exceedingly rare of Ph-negative MPDs is considered as an orphan disease with less known clinical presentation, prognosis, and challenging treatment. The multidisciplinary significance of SM can be attributed to the pathological accumulation of morphologically and immunophenotypically abnormal mast cells in one or more organ systems. From mast cell diseases (MCDs) our research focussed on systemic mastocytosis (SM) due to its low incidence rate, heterogeneous manifestation, and clinical complexity and the diagnostic difficulties often associated with it. As the presentation of SM may vary from asymptomatic to severe forms, its diagnosis can be especially challenging, both from a clinical and pathological perspective. Up to now, only very limited epidemiological data are available on SM. Although a small set of regional data have been collected through the European Competence Network on Mastocytosis (ECNM), no Hungarian data at all are present.
Among Ph-negative MPDs, hypereosinophilic syndromes (HESs) make up the most heterogeneous and widely debated group of diseases. The clinical presentation of HES is highly variable ranging from a relatively asymptomatic disease to endomyocardial fibrosis. Although HES may also be associated with other organ system failures, cardiac involvement and in particular Loeffler’s endocarditis still remain its best known manifestation. The cardiac involvement begins with eosinophilic infiltration, followed by an intermediate thrombotic stage, and finally evolving into a late fibrotic stage. Enlarged atrium with normal-sized left ventricle (LV) is a minor criterion for endomyocardial fibrosis. At this moment, little is known about left atrial (LA) function in HES. In our research, we investigated a) the ability of a novel non-invasive clinical tool (three-dimensional speckle tracking echocardiography, 3DSTE) to reveal any change in the cardiac functional parameters in a subtype of HES considered idiopathic in its etiology HES (iHES) without manifest organ damage (as determined by conventional diagnostic methods) as well as b) the left ventricular (LV) rotational mechanics in clinically symptomatic Loeffler’s endocarditis.

The diagnosis and treatment of all three Ph-negative MPDs require a strong multidisciplinary (cardiological – neurological – dermatological) approach and a close co-operation with other clinical specialists.

2.  AIMS

The aim of our research was to create a retrospective database of Ph-negative MPNs including three separate cohorts of patients diagnosed with PV, SM, or HES. This work evaluates and analyses separately the PV, SM, and HES patient cohorts of the 2nd Department of Internal Medicine and Cardiology Centre, Albert Szent-Györgyi Health Centre, Faculty of Medicine, University of Szeged, with the following objectives in each cohort:

Given that PV is the most common type of Ph-negative MPDs and in this group of diseases, vascular events are of outstanding significance and diagnostics, efficacious treatment can only be achieved on a multidisciplinary level, we assessed our patient population in the following aspects:

I.  PV cohort:

a) to evaluate the incidence of thrombotic events prior to and during follow-up; and to investigate the main clinical characteristics of PV patients, either with or without thrombotic complications in the follow-up period;

b) to assess the major cerebrovascular and cardiac thrombotic complications as the most serious thrombotic complications in the PV cohort; and to investigate whether any typical neurological or cardiac lesion(s) could be identified which might be specific to, or characteristic of PV; and

c) to evaluate the contribution of the main CV risk factors present at time of haematological diagnosis of PV as possible additional risk factors for subsequent thrombotic complications.

II.  SM cohort:

Analyses in our SM patient group were driven by the orphan nature and the diagnostic, therapeutic, and prognostic difficulties usually associated with this condition as follows:
a) to describe the clinicopathological findings along with bone marrow histological features, molecular characteristics, and laboratory parameters at presentation in a large cohort of SM patients; and to identify additional clinical, laboratory, and/or molecular genetic parameters with possible prognostic value;
b) to evaluate the frequency of KIT D816V mutation in SM subgroups;
c) to estimate the life expectancy of SM patients compared to age- and sex-matched controls; and to evaluate the prognostic relevance of the WHO classification of SM in the investigated patient population; and
d) to quantify the cumulative incidence of SM in the South Great Plain region of Hungary.

III. HES cohort:
HES, a condition with highly variable clinical presentation and organ involvement also belongs to the group of rare Ph-negative MPDs. In this patient population, our objectives were:

a) to compare LA volumetric, volume-based functional, and strain parameters obtained by three-dimensional speckle-tracking echocardiography (3DSTE), a novel, non-invasive clinical tool for volumetric and strain analysis, between HES patients and matched controls; and
b) to demonstrate LV rotational mechanics in a unique case with Loeffler's endocarditis.

3. PATIENTS, METHODS, AND RESULTS

As basis for this research, we retrospectively established a database for scientific research, focussing especially on PV, SM, and HES cases diagnosed at the 2nd Department of Internal Medicine and Cardiology Centre between 1998 and 2014. Our investigations were conducted with the approval of the Regional and Institutional Human Medical Biological Research Ethics Committee of the Albert Szent-Györgyi Health Centre, University of Szeged and in accordance with the Declaration of Helsinki principles. Written informed consent was not required from the subjects. The review of trial subjects’ relevant medical data was done by using MedSolution, the healthcare data management system of the Albert Szent-Györgyi Health Centre, Faculty of Medicine, University of Szeged.

3.1. POLYCYthaEMIA VERA

3.1.1 STUDY POPULATION AND DATA COLLECTION

To establish our PV database, we relied on two sources. First, we screened medical records (MedSolution) of patients presented at the Haematology Outpatient Unit, 2nd Department of Internal Medicine and Cardiology Centre with the orienting diagnosis of
different chronic myeloproliferative diseases, per appropriate ICD (International Classification of Diseases, ICD-10) codes and in a given time period; and second, we checked data of patients subject to molecular diagnostic testing for myeloproliferative disorders at the Department of Medical Genetics, Faculty of Medicine, University of Szeged. Finally, we set up an integrated database including the relevant elements of both data sources. For our research, medical records of a total of 933 patients were screened. Out of them, the definite diagnosis of PV fulfilling WHO criteria could be established in 108 cases.

Thrombotic events prior to and following the clinical diagnosis were retrospectively collected for each PV patient, with special focus on cardiovascular (AMI), cerebrovascular (stroke, TIA, vertebrobasilar insufficiency [VBI]), and venous thrombotic events (DVT, PE, splanchnic vein thrombosis). Data on CV risk factors present at time of PV clinical diagnosis including hypertension, tobacco use, diabetes mellitus (DM), hyperlipidaemia (hypercholesterolaemia or hypertriglyceridaemia or both), and obesity (body mass index [BMI] >30 kg/m²) were also collected.

3.1.2 STATISTICAL ANALYSES

Clinical data were collected using Microsoft® Excel® 2010 software and subjected to statistical analysis with STATISTICA v9.1 (StatSoft, Hungary) and SPSS 20 (IBM, USA) softwares.

Investigation of the contribution of cardiovascular risk factors in polycythaemia vera

To evaluate and compare the overall effect of CV risk factors present at time of haematological diagnosis, Mann–Whitney U tests were performed. In addition to each predefined CV risk factor (hypertension, hyperlipidaemia, tobacco use, DM, and obesity), the effect of only one CV risk factor, the effect of two or more CV risk factors as well as the role of leukocytosis (>11.1 G/L) or increased Hct (>45%) were also investigated. Statistical significance was set at 5% and, as reasoned by study population size, also considered at 10%. To evaluate and compare the probability of thrombosis-free survival for PV patients without CV risk factors and with at least one CV risk factor and with at most one CV risk factor and with two or more CV risk factors, the Kaplan–Meier method was used, combined with log-rank (Mantel–Cox) tests.

3.1.3 LABORATORY METHODS

Samples for JAK2 V617F molecular analyses were obtained from the DNA bank of the Department of Medical between 1998 and 2014. Serum erythropoietin levels were determined by chemiluminescent immunoassay (Siemens Immulite) at the Laboratory of Endocrinology, University of Szeged.

3.1.4 RESULTS

Thrombotic events prior to and during follow-up and the main clinical characteristics of polycythaemia vera patients with or without thrombotic complications in the follow-up period (Aim I.a)

The retrospective analysis of all recorded events revealed altogether 33 pre-diagnosis vascular events in 108 (30.5%) patients: 17 cerebrovascular events (stroke/TIA), 8 CV events (AMI), and 8 venous thrombotic events. During the haematological follow-up after the diagnosis of PV, a total of 20 events were observed in 108 (18.5%) patients: 11 cerebrovascular events (stroke/TIA), 7 CV events (AMI), and 2 venous thrombotic events.
We investigated whether subjects with or without a post-diagnostic history of thrombotic events significantly differ in their main clinical characteristics. Mann-Whitney tests were performed in the cases of the presence or absence of thrombotic events after the diagnosis of PV, comparing the overall effects of series variables: mean follow-up, mean age at diagnosis, JAK2 V617F-positivity, haematology blood test results, organomegaly, number of thrombotic events before haematological diagnosis, vascular risk factors, and treatment. Fisher’s exact test results were considered for sex and conventional risk groups. Some CV risk factors as hypertension (p=0.001) and tobacco use (p=0.023) are significantly different in our two patient groups; and the different thrombotic risk of conventional low- and high-risk groups (p=0.029) was confirmed.

CARDIOVASCULAR AND CEREBROVASCULAR COMPLICATIONS (Aim I.b)

Cardiovascular complications

Detailed clinical data and coronary angiography findings for adequate cardiological analyses of CV complications in PV patients during follow-up were available for 6 patients (1 male, 5 females; mean age 69.5 years [range: 64–76 years]). Five (83.3%) out of the six analyzed patients exhibited JAK2 V617F mutation. Most of the patients (83.3%) had at least two major conventional CV risk factors. Non–ST-segment elevation myocardial infarction (NSTEMI) was diagnosed in all 6 PV patients by coronary angiography. Evaluated cases are listed individually in the dissertation.

Cerebrovascular complications

Detailed analysis of cerebrovascular events was performed in 11 out of the 108 PV patients enrolled (9 males, 2 females; median age: 65 years [range: 52–79 years]). JAK2 V617F mutation positivity was detected in 10 of the 11 analyzed cases (91%). Most of the patients (8/11, 72%) had at least two major conventional vascular risk factors.

In most of the cases (7/11 patients, 63%), chronic ischaemic white matter lesions were detected on brain computed tomography scan obtained at cerebrovascular event onset. The clinical presentation was predominated by lacunar syndromes or VBI. Two patients – one of them on anticoagulant therapy – sustained haemorrhagic stroke. Overall, these data allow us to suppose that after adjusting for major conventional vascular risk factors, PV predisposes to small vessel cerebral disease manifested mainly as lacunar syndromes, even if most of the patients had additional vascular risk factors, too. Evaluated cases are listed individually in the dissertation.

THE CONTRIBUTION OF CARDIOVASCULAR RISK FACTORS TO SUBSEQUENT THROMBOTIC COMPLICATIONS (Aim I.c)

Univariate analyses revealed a significant overall association between thrombotic complications and high blood pressure (p=0.000), tobacco use (p=0.014), and obesity (p=0.078). Hyperlipidaemia (p=0.112) and DM (p=0.323) were not associated with an increased risk of subsequent thrombosis. The presence of one CV risk factor (p=0.016) or two or more CV risk factors (p=0.024) significantly increased the occurrence of thrombotic complications. Leukocytosis (WBC >11.1 G/L), however, did not increase significantly the risk of thrombotic events (p=0.119). The frequency of thrombotic events during follow-up differed significantly between PV subgroups with Hct values below or above 45% (p=0.089). To compare the thrombosis-free survival of patients in the presence or absence of the investigated CV risk factors, Kaplan–Meier curves and log-rank tests (Mantel–Cox) were
used which indicated a significant difference of the thrombosis-free survival between PV patients without CV risk factors (N=20) and those with at least one CV risk factor (N=88) (p=0.017). A significant difference was also observed between PV patients with at most one CV risk factor (N=49) and PV patients with two or more CV risk factors (N=59) (p=0.011).

3.2. SYSTEMIC MASTOCYTOSIS

3.2.1 PATIENTS AND DATA COLLECTION

Between 2001 and 2013, a total of 35 patients were diagnosed with SM (20 males, 15 females; median age: 57 years [range: 31–85 years]) in our centre: 14 with ISM, 15 with SM-AHNMD, and 6 with ASM subtypes, respectively. In the investigated period, no other MCD entities like MCL, extracutaneous mastocytoma, or MCS occurred. Out of all registered cases, only those who strictly fulfilled the 2008 WHO criteria for SM (major criterion and ≥1 minor criterion, OR ≥3 minor criteria) were considered to be enrolled in our research.

3.2.2 STATISTICAL ANALYSES

Evaluation of the survival probability in systemic mastocytosis

The survival probability in the various SM subgroups (ISM, AHNMD, ASM) was estimated by Kaplan–Meier analyses. Calculated patient survival rates were compared to the expected survival data of age- and sex-matched Hungarian population controls obtained from the Hungarian Central Statistical Office. For each year, the incidence rate was calculated as the number of new cases divided by the mid-year population size. The latter was obtained as the mean of the population sizes on 1st January of the relevant year and the next year. The cumulative incidence for 13 years was computed as

\[ CI = 1 - e^{-\sum_{i=1}^{13} IR_i \cdot t_i} \]

where \( IR_i \) denotes the yearly incidence rates from the first to the thirteenth year, and \( t_i \) denotes the length of each time period which is one year in this case for all the 13 periods.

3.2.3 LABORATORY METHODS

All bone marrow test results released by the Laboratory of Tumour Pathology and Molecular Diagnostics, Szeged since 2001 were screened for SM and re-assessed by morphological, immunohistochemical and molecular (PCR) methods, according to the 2008 WHO criteria. We considered only those cases as SM which strictly fulfilled the relevant 2008 WHO criteria (major criterion and ≥1 minor criterion, OR ≥3 minor criteria). In Hungary, serum tryptase determination is currently not available; however, all our presented cases met the 2008 WHO criteria, even without known tryptase levels. \( C-KIT \) mutation analysis is routinely performed on every bone marrow biopsy sample evaluated at our centre. \( C-KIT \) mutations detected by PCR method in this patient population were confirmed by Sanger sequencing.
3.2.4 RESULTS

CLINICOPATHOLOGICAL FINDINGS ALONG WITH BONE MARROW HISTOLOGICAL FEATURES, MOLECULAR CHARACTERISTICS, AND LABORATORY PARAMETERS AT PRESENTATION IN A LARGE COHORT OF SYSTEMIC MASTOCYTOSIS PATIENTS (Aim II.a)

In our regional centre, 14 ISM, 15 AHNMD, and 6 ASM cases were diagnosed in the period between 2001 and 2013; MCL was, however, not detected in any patient. As our study aimed at the evaluation of histological features, molecular characteristics as well as laboratory and clinical parameters of adult SM, mastocytosis cases in paediatric patients or those in adults confined only to the skin (without available bone marrow biopsy results) were not included in our research. All bone marrow test results since 2001 were screened for SM and re-assessed by morphological, immunohistochemical and molecular (PCR) methods, according to the 2008 WHO criteria.

In the ISM group, bone marrow biopsy analysis revealed ISM in 14 patients (8 males and 6 females) with a median age of 55 years (range: 31–81 years). The median duration of follow-up was 50.5 months (range: 5–240 months). Cutaneous manifestation (UP) was detected in 57% (8/14) of the patients while mediator-related symptoms occurred in 28% (4/14) of them. 78% (11/14) of the ISM patients were positive for \( KIT \) \( D816V \) mutation.

A total of 15 patients (7 males and 8 females; median age: 57 years [range: 34–72 years]) were diagnosed with AHNMD. The median follow-up time in this subgroup was 25 months (range: 1–104 months). Bone marrow biopsy was done as required by signs of the associated neoplasm such as bone lesions or clinically significant peripheral blood count abnormalities, e.g. eosinophilia or elevated or decreased WBC or PLT counts. This subtype of SM was associated with MDS or AML in three cases each, with MF in two cases, or with ET, PV, CML, HES, multiple myeloma, peripheral T-cell lymphoma, or B-cell acute lymphoblastic leukaemia in one case each. In these patients, no cutaneous lesions or mediator-related symptoms were observed. \( KIT \) \( D816V \) mutation positivity was detected in 80% (12/15) of the patients.

ASM was diagnosed in 6 patients (4 males and 2 females) with a median age of 65 years (range: 54–85 years). Bone marrow biopsy was performed for hepatosplenomegaly with or without pancytopenia/anaemia/eosinophilia and weight loss. All ASM patients presented with at least one C finding, as defined by the 2008 WHO criteria (marked cytopenia, osteolysis with or without pathological fractures, ascites and elevated liver enzymes, malabsorption with hypoalbuminaemia, palpable splenomegaly with hypersplenism). Cutaneous lesions were detected in 33% (2/6) of the patients. Mediator-related symptoms occurred in one case. \( KIT \) \( D816V \) mutation positivity was confirmed in 83% (5/6) of the patients. The median follow-up was 20.5 months (range: 2–35 months).

Clinical symptoms, immunophenotypes as well as PCR and Sanger sequencing results for each patient are summarized in dissertation.

FREQUENCY OF \( KIT \) \( D816V \) MUTATION (Aim II.b)

\( KIT \) \( D816V \) mutation positivity was detected in 78% (11/14) of ISM patients, 80% (12/15) of AHNMD patients and 83% (5/6) of ASM patients, respectively.

LIFE EXPECTANCY IN SYSTEMIC MASTOCYTOSIS (Aim II.c)

Overall disease-specific survival of SM patients was analyzed by Kaplan–Meier method.
The median survival in the ASM group was 1.73 years while the survival time of the AHNMD patients did not reach a median during follow-up. None of the patients died during the follow-up period in the ISM subpopulation. The median survival for the age- and sex-matched control population was 23.5 years. The comparison of the survival curves using Mantel–Cox, Breslow and Tarone–Ware tests uniformly resulted in a p-value of 0.000 indicating significantly different survival patterns in the evaluated SM subgroups.

Moreover, it is important to emphasize that our research identified an uncommon case in the investigated patient population. In our case report we demonstrated a female ISM patient with recurrent cutaneous symptoms and a follow-up time of 27 years. This case illustrates that in selected cases, imatinib mesylate could be a good choice to achieve a reduction of skin lesions, like in this KIT D816V-negative patient. (List of publications related to the thesis IV: Imelda Marton et al.: Therapeutic challenge during the long-term follow-up of a patient with indolent systemic mastocytosis with extensive cutaneous involvement.)

Cumulative Incidence of Systemic Mastocytosis (Aim II.d)

Our regional diagnostic and treatment centre receives SM patients from the population of south-eastern Hungary, representing a total of 1,103,463 inhabitants [56]. These data allowed us to calculate the cumulative incidence of SM for 13 years in the general population (aged 15 years or more), which proved to be 0.27/10,000 in this region.

3.3. Hyperesinophilic Syndrome

3.3.1 Patients and Data Collection in the Hyperesinophilic Syndrome Population

The diagnosis of idiopathic HES can only be established after the exclusion of all primary and secondary causes of hypereosinophilia and lymphocyte-variant hypereosinophilia. We evaluated 10 iHES patients with hypereosinophilia fulfilling the 2008 WHO criteria but without any secondary causes or underlying clonal disease. All patients were asymptomatic; none of them had a known Loeffler’s endocarditis. Cytogenetic, FISH, and molecular analyses of FIP1L1–PDGFRα were negative for all subjects in this population of 10 iHES patients without any underlying disease.

Due to the presence of Loeffler’s endocarditis and the rare neurological complication, the case of the patient with FIP1L1–PDGFRα mutation will be discussed separately. (List of publications related to the thesis VI: Imelda Marton et al.: Watershed infarction in hypereosinophilic syndrome: a diagnostic dilemma in FIP1L1-PDGFRα-associated myeloid neoplasm and overview of the relevant literature)

3.3.2 Statistical Analyses

All HES patients and their age- and sex-matched healthy controls underwent complete two-dimensional (2D) Doppler echocardiography and 3DSTE. Data of altogether 10 HES patients were compared to matching data of 19 control subjects.

Continuous variables were calculated as mean ± standard deviation. All statistical tests were two-sided. The cut-off value for statistical significance was set at p=0.05. Continuous parameters were compared using unpaired Student’s t test while categorical variables were
analyzed by chi-square or Fischer’s exact test. The correlation was defined by Pearson’s correlation coefficient. Statistical evaluations were performed using MedCalc software (MedCalc Inc., Mariakerke, Belgium).

3.3.3 METHODS – LABORATORY TESTS AND ECHO CARDIOGRAPHY

Diagnostic bone marrow samples of all patients investigated for hypereosinophilia were evaluated at our local tumour pathology laboratory. Cytogenetic tests were completed at the laboratory for cytogenetics of the 2nd Department of Internal Medicine and Cardiology Centre while FISH assays were performed at Department of Hematology and Stem Cell Transplantation, St. Istvan and St. Laszlo Hospital of Budapest, Molecular genetic tests for FIP1L1–PDGFRA were carried out in a close co-operation with the team of the Laboratory of Molecular Diagnostics, Hungarian National Blood Transfusion Service, Budapest [63]

Three-dimensional speckle-tracking echocardiography (3DSTE)

3DSTE datasets were acquired from apical window using the 1–4 MHz matrix phased-array transducer (PST-25SX) [78]. Following gain setting optimization, full volume mode was used over six consecutive cardiac cycles during a single breath-hold. Volume data were stored in raw data format for further analysis. LA quantifications were performed using 3D Wall Motion Tracking software v2.7 (Toshiba Medical Systems, Tokyo, Japan). Each 3D dataset was displayed in multiple plane views including the apical two- (AP2CH) and four-chamber (AP4CH) views and three short-axis views at different LA levels from the base to the apex. Several reference points on the LA endocardium were set by the examiner in the AP2CH and AP4CH views. The first points were set at the edge of the septal mitral valve ring where anterior mitral leaflet origins and then markers were placed in a counterclockwise rotation around the LA to the lateral mitral valve ring (to the origin of the posterior leaflet) in the AP4CH viewing plane. During evaluations, LA appendage and the pulmonary veins were excluded from the LA cavity. Measurements were performed first on AP4CH view and then on AP2CH view. After detection of the LA myocardial borders at the end-diastolic reference frame, the user could correct the LA shape if necessary. The 3D wall motion tracking was then automatically performed through the entire cardiac cycle.

3DSTE for left atrial volumetric measurements

From time curves of global LA volume changes, maximum (V_max), minimum LA volumes (V_min) and LA volume before atrial contraction (V_preA) were measured using the 3D echocardiographic datasets just before mitral valve opening (end-systole), just before mitral valve closure (end-diastole) and at time of P-wave on electrocardiography (early diastole), respectively [78-82] LA function consists of three phases: the systolic reservoir phase and the diastolic passive (conduit) and active emptying (booster pump) phases. To characterize these functions, stroke volumes (SVs) and emptying fractions (EFs) were calculated from the above-mentioned volumes as follows:

Left atrial stroke volumes
- Total Atrial Stroke Volume (TASV): V_max–V_min (reservoir function)
- Passive Atrial Stroke Volume (PASV): V_max–V_preA (conduit function)
- Active Atrial Stroke Volume (AASV): \( V_{\text{pre}A} - V_{\text{min}} \) (booster pump/active contraction function)

**Left atrial emptying fractions**
- Total Atrial Emptying Fraction (TAEF): \( \frac{TASV}{V_{\text{max}}} \times 100 \) (reservoir function)
- Passive Atrial Emptying Fraction (PAEF): \( \frac{PASV}{V_{\text{max}}} \times 100 \) (conduit function)
- Active Atrial Emptying Fraction (AAEF): \( \frac{AASV}{V_{\text{pre}A}} \times 100 \) (booster pump/active contraction function)

**3DSTE for left atrial strain measurements**

From the same 3D echocardiographic datasets, time curves of unidirectional radial (RS), longitudinal (LS), and circumferential strains (CS) were also generated for each segment using the 16-segment model obtained for LV [81-84]. Moreover, due to the ability of 3DSTE to calculate complex strains, area strain (AS, as a ratio of endocardial area change during cardiac cycle) and three-dimensional strain (3DS, strain in the direction of wall thickening, combination of “unidirectional” strains) were also measured. At each time, segmental strain curve peak strains representing characteristics of reservoir phase of the LA function were measured. Global strains were calculated by the software considering the whole LA while mean segmental strains were obtained as the average of strains of 16 segments as well. These parameters were calculated automatically by the software.

**3.3.4 RESULTS**

**Comparison of left atrial volumetric, volume-based functional, and strain parameters obtained by three-dimensional speckle-tracking echocardiography, a novel, non-invasive clinical tool for volumetric and strain analysis, between patients with hyperesinophilic syndrome and matched controls (Aim III.a)**

No correlation was found between any of the laboratory findings and 2D echocardiographic or 3DSTE data in this patient population. None of the control and HES patients exhibited >Grade 1 mitral or tricuspid regurgitation. Significant difference was only found in LA diameter and interventricular septum thickness between HES and control subjects. Only one patient had a prior cardiac disease (NSTEMI) in his history. Clinical and 3DSTE data of each patient are presented in dissertation. Both global and mean segmental peak CS were significantly reduced in HES patients suggesting an impaired LA reservoir function. Significantly increased maximum (\( p=0.01 \)) and minimum (\( p=0.03 \)) LA volumes as well as LA volume before atrial contraction (\( p=0.01 \)) and elevated total (\( p=0.02 \)) and active (\( p=0.005 \)) atrial SVs values characterizing reservoir and booster pump LA function were found in HES patients, as compared to controls. EF did not significantly differ between groups.

**Presentation of left ventricular rotational mechanics through a unique case of hyperesinophilic syndrome with Loeffler’s endocarditis by means of the novel method of 3DSTE (Aim III.b)**

Loeffler’s disease is associated with stiffened ventricular and atrial walls leading to inadequate filling, decreased preload, diastolic dysfunction, and heart failure. At this moment, little is known about the rotational characteristics of the Loeffler’s heart. Therefore, we present a 36-year-old male patient with known Loeffler’s endocarditis on optimal therapy whose rotational parameters were evaluated by 3DSTE. Due to its unique nature and potential
clinical relevance, we demonstrated it in a separate case report, providing therefore an additional proof of the complexity of HES. (List of publications related to the thesis VII.: Attila Nemes, Anita Kalapos, Péter Domsik, Imelda Marton, Zita Borbenyi, Tamás Forster: Three-dimensional speckle-tracking echocardiography in Loeffler endocarditis: case report from the MAGYAR-Path Study)

4. SUMMARY

CONCLUSIONS AND CLINICAL IMPLICATIONS OF OUR RESEARCH

For purposes of our research focussing especially on Ph-negative MPNs, we retrospectively established a database of PV, SM, and HES cases diagnosed at the 2nd Department of Internal Medicine and Cardiology Centre between 1998 and 2014. The collected data were analyzed and assessed by disease-relevant factors and in accordance with literature requirements.

Polycythaemia vera

In PV, the most common type of Ph-negative MPDs our research focussed on vascular complications. Vascular events represent the primary cause of morbidity and mortality in PV and require therefore a strong multidisciplinary approach in their diagnosis and treatment.

- The number of major thrombotic events in PV patients that occurred prior to (30.32%) or during the follow-up (14.83%) was evaluated retrospectively.
- We conducted a separate analysis and evaluation of each and every CV and cerebrovascular event which are usually presented as aggregate data in large-scale trials.
- Regarding cerebrovascular complications in PV, in our cohort of PV patients, the majority of cerebrovascular complications were chronic ischaemic white matter lesions. Mild cerebral atrophy was also a frequent finding. The clinical presentation of cerebrovascular events was predominated by lacunar syndromes or VBI. Most of the patients presented at least two serious conventional vascular risk factors, which were supposed to have an impact on both the clinical course of the disease and the morphological alterations seen on brain imaging. These findings suggest that PV predisposes to small vessel cerebral disease manifested primarily as lacunar syndromes, despite the simultaneous presence of additional vascular risk factors in most patients.
- Regarding cardiovascular complications, NSTEMI was observed. Most patients had at least two conventional vascular risk factors. Our findings led us to suppose that the early diagnosis followed by percutaneous coronary intervention and an aggressive and personalized management of CV risk factors may be effective in the prevention of subsequent vascular events. The importance of a close co-operation between the haematologist and specialists in the field of vascular medicine is emphasized.
- The contribution of CV risk factors as newly hypothesized additional risk factors to subsequent thrombotic complications in PV was demonstrated in our patient population. Our findings clearly indicate the importance of the identification and consideration of these risk factors in a more accurate and individualized risk-guided thrombosis management in PV.
Systemic mastocytosis

Analyses in our SM patient group were driven by the orphan nature and the diagnostic, therapeutic, and prognostic difficulties usually seen in this condition. The multidisciplinary significance of SM lies in its challenging diagnostic aspects and characteristic multi-organ nature.

- As part of our research, we analyzed bone marrow histological features, molecular characteristics, and laboratory and clinical parameters at presentation in a large cohort of SM patients.
- The frequency of \( KIT \, D816V \) mutation in our study population was evaluated by SM subtype. The established occurrence of this particular mutation and its impact on the clinicohaematological findings was compliant with general literature data.
- Life expectancy of SM patients was compared to age- and sex-matched controls. In addition, the prognostic relevance of the 2008 WHO classification of SM in the investigated patient population was evaluated. Our analyses on Hungarian patients revealed that the survival in SM (including all subtypes) is worse than that expected in the age- and sex-matched Hungarian population. The life expectancy of patients with ISM was excellent whereas SM-AHNMD and ASM groups had a reduced median survival. The distribution of subtypes and the survival pattern seen in our SM study population was similar to that reported in the largest trial previously published.
- Epidemiological data on SM, an orphan Ph-negative MPN are only sparsely available in the literature. With our research, we provided important new data on SM and quantified its cumulative incidence in the South Great Plain region of Hungary.

Hypereosinophilic syndrome

HES is a particularly heterogeneous entity with a wide range of clinical manifestations and a typically multi-organ presentation, facilitating a multidisciplinary thinking in its management.

- We first performed investigations with 3DSTE, a novel non-invasive cardiac diagnostic tool in patients with HES. All HES patients and their age- and sex-matched healthy controls underwent complete 2D Doppler echocardiography and 3DSTE.
- On 3DSTE, increased LA volumes and LA stroke volumes were demonstrated in HES patients, accompanied by reduced LA-CS values. These findings suggest a structural and functional LA remodelling in the evaluated patients.
- The diagnostic work-up requires a close and effective multidisciplinary co-operation between the cardiologist, neurologist, the neuroradiologist, and the haematologist in order to achieve an early and precise diagnosis and the successful management of cases with hypereosinophilic syndrome.

The diagnosis and treatment of all three investigated Ph-negative MPDs is highly complex, calling for a multidisciplinary collaboration among several clinical and non-clinical divisions.
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