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American Society of Hematology
2021 L Street NW, Suite 900,
Washington, DC 20036
Phone: 202-776-0544 | Fax 202-776-0545
bloodadvances@hematology.org

Serum chemistry profiling and prognostication in systemic mastocytosis: a registry-based study of the ECNM and GREM

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Johannes Lübke (University Hospital Mannheim, Heidelberg University, Germany) Alicia Schmid (University Hospital Mannheim, Heidelberg University, Germany) Deborah Christen (Department of Oncology, Haematology, Haemostaseology and Stem Cell Transplantation, University Hospital RWTH Aachen, Center for Integrated Oncology Aachen Bonn Cologne Duesseldorf (CIO ABCD), Germany, Germany) Hanneke N.G. Oude Elberink (University Medical Center Groningen, Netherlands) Lambertus Span (University Medical Center Groningen, Netherlands) Marek Niedoszytko (Medical University of Gdansk, Poland) Aleksandra Górska (Department of Allergology, Medical University of Gdańsk, Poland) Magdalena Lange (Medical University of Gdansk, Poland) Karoline Gleixner (Medical University of Vienna, Austria, Austria) Emir Hadzijusufovic (Medical University of Vienna, Austria) Alex Stefan (Johannes Kepler University, Austria) Irena Angelova-Fischer (Kepler University Hospital, Austria) Roberta Zanotti (IRCSS Ospedale Sacro Cuore Don Calabria, Italy) Massimiliano Bonifacio (UNIVERSITY OF VERONA, Italy) Patrizia Bonadonna (Allergy Unit, Italy) Khalid Shoumariyeh (Department of Medicine I, Medical Center - University of Freiburg, Faculty of Medicine, University of Freiburg, Germany, Germany) Nikolas von Bubnoff (Universitätsklinikum Schleswig-Holstein, Campus Lübeck, Germany) Sabine Müller (Department of Dermatology, Medical Center, University of Freiburg, Faculty of Medicine, University of Freiburg, Germany) Cecelia Perkins (Stanford Cancer Institute, United States) Chiara Elena (Foundation IRCCS policlinico san matteo, Italy) Luca Malcovati (University of Pavia, Italy) Hans Hagglund (Dept of Medical Sciences, Sweden) Mattias Mattsson (Department of Immunology, Genetics and Pathology, Sweden) Roberta Parente (University of Salerno, Italy) Judit Varkonyi (Semmelweis University, Hungary) Anna Fortina (University of Padova, Italy) Francesca Caroppo (Dermatology Unit, University of Padova, Italy) Knut Brockow (School of Medicine, Technical University of Munich, Germany) Alexander Zink (School of Medicine, Technical University of Munich, Germany) Christine Breynaert (KU Leuven/University Hospitals Leuven, Belgium) Toon Ieven (ven Department of Microbiology, Immunology and Transplantation, Belgium) Akif Yavuz (Istanbul University, Istanbul Faculty of Medicine, Turkey) Michael Doubek (Department of Internal Medicine - Hematology and Oncology, University Hospital Brno & Faculty of Medicine, Masaryk University, Czech Republic) Vito Sabato (Antwerp University Hospital, Belgium) Tanja Schug (Med. Univ. Graz, Univ.Klinik für Dermatologie und Venereologie, Austria) Karin Hartmann (University Hospital Basel and University of Basel, Switzerland) Massimo Triggiani (University of Salerno, Italy) Jason Gotlib (American Society of Hematology, United States) olivier hermine (INSERM U1163 CNRS ERL8254 Imagine Institute, France) Michel Arock (Pitié-Salpêtrière Hospital, France) Hanneke Kluin-Nelemans (University Medical Center Groningen, University og Groningen, Netherlands) Jens Panse (University Hospital, RWTH Aachen, Germany) Wolfgang Sperr (Dept. of. Int.Med.I, Div.of Hematology, Austria) Peter Valent (Medical University of Vienna, Austria) Andreas Reiter (University Hospital Mannheim, Heidelberg University, Germany) Juliana Schwaab (III.Medizinische Klinik, Germany)

Abstract:
Certain laboratory abnormalities correlate with subvariants of systemic mastocytosis (SM) and are often prognostically relevant. To assess the diagnostic and prognostic value of individual serum chemistry parameters in SM, 2607 patients enrolled within the European Competence Network on Mastocytosis (ECNM) and 575 patients enrolled within the German Registry on Eosinophils and Mast Cells (GREM) were analyzed. For screening and diagnosis of SM, tryptase was identified as the most specific serum parameter. For differentiation between indolent and advanced SM (AdvSM), the following serum parameters were most relevant: tryptase, alkaline phosphatase (AP), ß2-microglobulin, lactate dehydrogenase (LDH), albumin, vitamin B12, and C-reactive protein (P<0.001). With regard to subvariants of AdvSM, an elevated LDH {greater than or equal to}260U/L was associated with multi-lineage expansion (leukocytosis, r=0.37, P<0.001; monocytosis, r=0.26, P<0.001) and the presence of an associated myeloid neoplasm (P<0.001), whereas tryptase levels were highest in mast cell leukemia (MCL vs. non-MCL, 308 µg/L vs. 146 µg/L, P=0.003). Based on multivariable analysis, the hazard-risk weighted assignment of 1 point to lactate dehydrogenase (HR 2.1 [95% CI 1.1-4.0], P=0.018) and 1.5 points each to ß2-microglobulin (HR 2.7 [95% CI 1.4-5.4], P=0.004) and albumin (HR 3.3 [95% CI 1.7-6.5], P=0.001) delineated a highly predictive three-tier risk classification system (0 points, 8.1 years vs. 1 point, 2.5 years, {greater than or equal to}1.5 points, 1.7 years; P<0.001). Moreover, serum chemistry parameters enabled further stratification of IPSM-AdvSM1/2 risk-score classified patients (P=0.027). In conclusion, serum chemistry profiling is a crucial tool in the clinical practice supporting diagnosis and prognostication of SM and its subvariants.-

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Serum chemistry profiling and prognostication in systemic mastocytosis: a registry-based study of the ECNM and GREM

Johannes Lübke, MD[1], Alicia Schmid, MD[1], Deborah Christen, MD[2,3], Hanneke N.G. Oude Elberink, MD[4], Lambert F.R. Span, MD, PhD[5], Marek Niedoszytko, MD[6], Aleksandra Gorska, MD[6], Magdalena Lange, MD[7], Karoline V. Gleixner, MD[8,9], Emir Hadzijusufovic, DVM[8,9,10], Alex Stefan, MD[11], Irena Angelova-Fischer, MD, PhD[12], Roberta Zanotti, MD[13], Massimiliano Bonifacio, MD[13], Patrizia Bonadonna, MD[14], Khalid Shoumariyeh, MD[15,16], Nikolas von Bubnoff, MD[15,16,17], Sabine Müller, MD[18], Cecelia Perkins, MH[19], Chiara Elena, MD[20], Luca Malcovati, MD[20], Hans Hagglund, MD[21], Mattias Mattsson, MD[21], Roberta Parente, MD, PhD[22], Judit Varkonyi, MD[23], Anna Belloni Fortina, MD[24], Francesca Caroppo, MD[24], Knut Brockow, MD[25], Alexander Zink, MD[25], Christine Breynaert, MD[26], Toon Leven, MD[26], Akif Selim Yavuz, MD[27], Michael Doubek, MD[28], Vito Sabato, MD[29], Tanja Schug, MD[30], Karin Hartmann, MD[31,32], Massimo Triggiani, MD, PhD[22], Jason Gotlib, MD, MS[19], Olivier Hermine, MD PhD[33], Michel Arock, PharmD[34], Hanneke C. Kluin-Nelemans, MD[5], Jens Panse, MD[2,3], Wolfgang R. Sperr, MD, PhD[8,9], Peter Valent, MD[8,9], Andreas Reiter, MD[1], Juliana Schwaab, MD[1]

1. Department of Hematology and Oncology, University Hospital Mannheim, Heidelberg University, Mannheim, Germany;
2. Department of Oncology, Haematology, Haemostaseology and Stem Cell Transplantation, University Hospital RWTH Aachen, Aachen, Germany;
3. Center for Integrated Oncology, Aachen Bonn Cologne Düsseldorf (CIO ABCD), Aachen, Germany;
4. Department of Allergology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands;
5. Department of Hematology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands;
6. Department of Allergology, Medical University of Gdansk, Gdańsk, Poland;
7. Department of Dermatology, Venereology and Allergology, Medical University of Gdansk, Poland;
8. Department of Internal Medicine I, Division of Hematology & Hemostaseology, Medical University of Vienna, Austria;
9. Ludwig Boltzmann Institute for Hematology and Oncology, Medical University of Vienna, Austria;
10. Internal Medicine Small Animals, University Clinic for Small Animals, Department/University Clinic for Companion Animals and Horses, University of Veterinary Medicine, Vienna, Austria;
11. University Clinic for Hematology and Oncology, Kepler University Hospital, Johannes Kepler University, Linz, Austria;
12. Department of Dermatology and Venereology, Allergy Center, Kepler University Hospital, Johannes Kepler University Linz, Austria.
13. Section of Hematology, Department of Medicine, Verona University Hospital, Verona, Italy;
14. Allergy Unit, Verona University Hospital, Verona, Italy;
15. Department of Medicine I, Medical Center - University of Freiburg, Faculty of Medicine, University of Freiburg, Germany;
16. German Cancer Consortium (DKTK), partner site Freiburg and German Cancer Research Center (DKFZ), Heidelberg, Germany
17. Department of Hematology and Oncology, Medical Center, University of Schleswig-Holstein, Campus Lübeck, Lübeck, Germany;
18. Department of Dermatology, Medical Center, University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany;
19. Stanford Cancer Institute/Stanford University School of Medicine/Stanford Cancer Institute, Stanford, California, United States;
20. Hematology Unit, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy;
21. Division of Hematology, Department of Medical Sciences Uppsala University, Uppsala, Sweden;
22. Division of Allergy and Clinical Immunology, University of Salerno, Salerno, Italy;
23. Department of Hematology, Semmelweis University, Budapest, Hungary;

24. Pediatric Dermatology Unit, Department of Medicine, University of Padova, Padova, Italy;
25. Department of Dermatology and Allergy, School of Medicine, Technical University of Munich, Munich, Germany;
26. KU Leuven Department of Microbiology, Immunology and Transplantation, Allergy and Clinical Immunology Research Group and MASTeL, University Hospitals Leuven, Leuven, Belgium
27. Division of Hematology, Istanbul Medical School, University of Istanbul, Istanbul, Turkey;
28. University Hospital and Faculty of Medicine, Brno, Czechia;
29. Faculty of Medicine and Health Sciences, Department of Immunology-Allergology-Rheumatology, University of Antwerp and Antwerp University Hospital, Antwerpen, Belgium;
30. Department of Dermatology and Venereology, University Hospital Graz, Graz, Austria;
31. Division of Allergy, Department of Dermatology, University Hospital Basel and University of Basel, Basel, Switzerland;
32. Department of Biomedicine, University Hospital Basel and University of Basel, Basel, Switzerland
33. French Reference Center for Mastocytosis [1], Hospital Necker, Assistance Publique Hôpitaux de Paris, Imagine Institute, University Paris Descartes, Paris, France;
34. French Reference Center for Mastocytosis [1], Pitié-Salpêtrière Hospital, Assistance Publique Hôpitaux de Paris, University Paris Sorbonne, Paris, France

Corresponding author:
Juliana Schwaab, M.D.
Department of Hematology and Oncology
University Hospital Mannheim
Theodor-Kutzer-Ufer 1-3
68167 Mannheim
Germany
Tel.: +49-621-383-4214
Fax: +49-621-383-4201
E-Mail: Juliana.schwaab@medma.uni-heidelberg.de

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author (JS) on reasonable request.

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ABSTRACT

Certain laboratory abnormalities correlate with subvariants of systemic mastocytosis (SM) and are often prognostically relevant. To assess the diagnostic and prognostic value of individual serum chemistry parameters in SM, 2607 patients enrolled within the European Competence Network on Mastocytosis (ECNM) and 575 patients enrolled within the German Registry on Eosinophils and Mast Cells (GREM) were analyzed. For screening and diagnosis of SM, tryptase was identified as the most specific serum parameter. For differentiation between indolent and advanced SM (AdvSM), the following serum parameters were most relevant: tryptase, alkaline phosphatase (AP), ß2-microglobulin, lactate dehydrogenase (LDH), albumin, vitamin B12, and C-reactive protein (*P*<0.001). With regard to subvariants of AdvSM, an elevated LDH ≥260U/L was associated with multi-lineage expansion (leukocytosis, r=0.37, *P*<0.001; monocytosis, r=0.26, *P*<0.001) and the presence of an associated myeloid neoplasm (*P*<0.001), whereas tryptase levels were highest in mast cell leukemia (MCL vs. non-MCL, 308 µg/L vs. 146 µg/L, *P*=0.003). Based on multivariable analysis, the hazard-risk weighted assignment of 1 point to lactate dehydrogenase (HR 2.1 [95% CI 1.1-4.0], *P*=0.018) and 1.5 points each to ß2-microglobulin (HR 2.7 [95% CI 1.4-5.4], *P*=0.004) and albumin (HR 3.3 [95% CI 1.7-6.5], *P*=0.001) delineated a highly predictive three-tier risk classification system (0 points, 8.1 years vs. 1 point, 2.5 years, ≥1.5 points, 1.7 years; *P*<0.001). Moreover, serum chemistry parameters enabled further stratification of IPSM-AdvSM1/2 risk-score classified patients (*P*=0.027). In conclusion, serum chemistry profiling is a crucial tool in the clinical practice supporting diagnosis and prognostication of SM and its subvariants.

KEY POINTS

- Serum chemistry profiling is of diagnostic and prognostic relevance based on easily accessible parameters.
- Serum chemistry markers help to assess and quantify organ damage.

INTRODUCTION

Systemic mastocytosis (SM) is characterized by expansion and accumulation of neoplastic mast cells (MC) in various organ systems, frequently including the bone marrow (BM), skin and the gastrointestinal tract. According to the World Health Organization (WHO) 2022 and the International Consensus Criteria (ICC) 2022, main subvariants of SM are indolent SM (ISM), smoldering SM (SSM) and advanced SM (AdvSM). BM mastocytosis (BMM) has previously been defined as a provisional sub-variant of ISM, and is now recognized as a separate variant of SM by the WHO. BMM is characterized by a limited degree of BM infiltration, absence of skin lesions, normal or slightly elevated serum tryptase levels, older age, male predominance, and a strong association with severe allergic reactions to hymenoptera sting. AdvSM comprises aggressive SM (ASM), SM with an associated hematologic/myeloid neoplasm (SM-AHN/SM-AMN) and MC leukemia (MCL). In AdvSM, the majority of patients presents with signs of organ damage (C-findings), particularly cytopenias and signs of liver involvement.[1-4] A somatic point mutation in *KIT* at codon 816 (*KIT* D816V) is identified in >80% of all patients with SM.[5-8] While patients with indolent forms have a normal life expectancy, patients with AdvSM have a poor prognosis with shortened survival times ranging between 1 and 4 years.[9-14]

Serum chemistry parameters have been variably included in recently published risk-stratification scoring systems for SM. Besides serum tryptase, which is known for its outstanding value for screening and diagnosis, hypoalbuminemia and elevated alkaline phosphatase (AP) are established C-findings while ß2-microglobulin is an unspecific but valuable prognostic marker in the Global Prognostic Score for SM.[12,15,16]

Notwithstanding, thorough and systematic analyses on the diagnostic and prognostic value of those low-invasive and reproducible biomarkers are lacking. Based on data

obtained from two registries (European Competence Network on Mastocytosis, ECNM; German Registry on Eosinophils and Mast cells, GREM), we therefore sought to establish various serum chemistry parameters for diagnosis and prognostication, the differentiation between ISM and AdvSM and the differentiation between various subvariants of AdvSM.

PATIENTS AND METHODS

Patients. SM was diagnosed and classified according to the revised 2022 WHO/ICC classification. Patients´ characteristics are provided in the supplement (Table E1). The study design adhered to the tenets of the Declaration of Helsinki and was approved by the relevant institutional review boards of the participating centers. All patients gave written informed consent prior to registry participation.

ECNM registry. The ECNM registry was established in 2012 as a multidisciplinary, multi-national cooperative initiative to analyze basic clinical, laboratory and prognostic parameters in patients with cutaneous mastocytosis (CM) and SM.[17-19] Details about the ECNM registry have been published elsewhere.[19] For this analysis, we used data from a validated cohort updated in March 2019. Patients were enrolled from 26 centers in Europe (12 countries) and one center in the United States. We identified 2,607 SM patients with at least one entry regarding one of the following 5 serum chemistry parameters: serum tryptase, AP, β2-microglobulin, lactate dehydrogenase (LDH), and/or albumin at time of diagnosis. Three patients with mast cell sarcoma and 635 patients with mastocytosis in the skin were also excluded. The 2,607 patients were subtyped as ISM (n=1734, 66%), BMM (n=327, 13%) SSM (n=58, 2%), ASM (n=117, 4%), SM-AHN (n=326, 13%) and MCL (n=45, 2%).

Availability of data and disease characteristics of the overall population and a subgroup of patients with available outcome data (n=685) are presented in the **Online Repository Tables E1-2**.

GREM registry. First patients were enrolled in 2009. An updated cohort in May 2019 (n = 575, ISM, n=224, 39%; BMM, n=55, 10%; SSM, n=23, 4%; ASM, n=32, 5%; SM-AHN, n=233, 41%; MCL, n=8, 1%) was used for complementary analyses on parameters that were primarily not included in the ECNM database (AP isoenzymes, C-reactive protein [CRP], creatinine, vitamin B12). A patient flow diagram is provided as **Online Repository Figure E1**.

Statistical analyses. In this retrospective study, statistical analyses were performed on clinical, laboratory and molecular parameters that were obtained at the time of diagnosis/first referral to the servicing center and throughout the disease course. Differences in the distribution of continuous variables between categories were analyzed by the Mann-Whitney U test. For categorical variables, Fisher's exact test was used. Overall survival (OS) analysis was considered from the date of diagnosis to date of death for any reasons (event) or last documented visit. Deaths unrelated to disease progression were observed in <0.1% of the patients. Censoring was applied for patients who did not experience death or were lost to follow-up within the study period (type 1 right-censored data). OS probabilities were calculated by the Kaplan-Meier Method and compared by the log-rank test. For multivariable analysis of serum chemistry markers, a cox proportional hazard model was used. The proportional hazards assumption was tested by the correlation of scaled Schoenfeld residuals with time.[20] Power calculation for the comparison of survival curves between two groups were performed according to Freedman.[21] Receiver operating characteristic

(ROC) analyses with calculation of the area under the ROC curve (AUC) were performed to determine optimal cut-off values for the several laboratory parameters. The Youdens index was given as the summary measure of the ROC curve (sensitivity + specificity – 1). The Pearson and Spearman correlation coefficients were calculated to assess linear or monotonic relationships between two variables. *P* values were further adjusted by Holm-Bonferroni. In general, a test result with *P* less than 0.05 has been considered as statistically significant. Statistical analyses were performed using R version 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria). For graphical output, GraphPad Prism version 9 was utilized (GraphPad Software Inc., San Diego, CA, USA).

The study was approved by our local ethics committee led by Professor Harald Kl**?**ter (2020-593N) and all patients gave written informed consent.

RESULTS

ECNM registry-based analyses

Comparison of baseline serum parameters in ISM versus AdvSM. In AdvSM, significantly higher median serum levels were observed for tryptase (164µg/L vs. 29µg/L, *P*<0.001, Mann-Whitney U test), AP (162U/L vs. 71U/L, *P*<0.001, Mann-Whitney U test), ß2-microglobulin (3.6mg/L vs. 1.8mg/L, *P*<0.001, Mann-Whitney U test) and LDH (181U/L vs. 168U/L, *P*<0.001, Mann-Whitney U test) while significantly lower median serum levels were observed for albumin (39g/dL vs. 44g/dL, *P*<0.001, Mann-Whitney U test, **Figure 1; Online Repository Tables E1**). ROC analyses identified optimal cut-off values for discrimination between ISM and AdvSM for serum tryptase at 125µg/L, for AP at 150U/L, for ß2-microglobulin at 2.5mg/L, for LDH at 260U/L and for albumin at 34g/dL. In 649 patients with diagnosis of SM and available outcome data, the log-rank test identified all respective markers as adverse

prognostic variables regarding OS (**Table 1**). Assignment of 1 point each to 5 individual parameters allowed to establish a serum score through which low-, intermediate-, and high-risk patients can be defined (median OS 15.1 vs. 3.4 vs. 1.5 years, *P*<0.001, log-rank test). (**Figure 2A**). Multivariable analysis identified ß2-microglobulin (HR 2.7 [95% CI 1.4-5.4], *P*=0.004), lactate dehydrogenase (HR 2.1 [95% CI 1.1-4.0], *P*=0.018) and albumin (HR 3.3 [95% CI 1.7-6.5], *P*=0.001) of prognostic significance. Based on the multivariable analysis, we built a hazard risk-weighted score and applied 1 point to patients with lactate dehydrogenase ≥260U/L, and 1.5 points each to patients with ß2-microglobulin ≥2.5mg/L and albumin ≥34g/dL (**Figure 2B**).

Comparison of baseline serum parameters in patients with ISM, BMM and SSM. Median serum tryptase and AP levels were significantly higher in SSM (202µg/L, 138U/L) and ISM (32µg/L, 72U/L) versus BMM (21µg/L, 67U/L; *P*<0.001 for all comparisons, Mann-Whitney U test). SSM was further associated with higher ß2-microglobulin levels compared to BMM (2.3mg/L vs. 1.8mg/L, *P*=0.010, Mann-Whitney U test). No differences were seen regarding the median albumin level (44g/dL in all subgroups). Surprisingly, BMM (175U/L) showed higher LDH levels than SSM (153U/L, *P*=0.002) and ISM patients (167U/L, *P*=0.001, Mann-Whitney U test). Patients with serum tryptase levels ≥125µg/L were associated with an adverse median OS (15.1 vs. 9.7 years, *P*=0.045, log-rank test; **Online Repository Tables E1).**

Comparison of baseline serum parameters within subvariants of AdvSM. Serum tryptase levels were significantly higher in MCL (308µg/L) as compared to ASM (171µg/L, *P*=0.006, Mann-Whitney U test) and SM-AHN (135µg/L, *P*=0.002, Mann-

Whitney U test). An elevated LDH level ≥260U/L was associated with the presence of an AHN (77/236, 33% vs. 199/1715, 12%; *P*<0.001, Mann-Whitney U test) and with a more aggressive clinical course (Table 2, Figure 3B). The median LDH levels were significantly higher in SM-AHN than in ASM (191 U/L vs. 162U/L, *P*<0.001, Mann-Whitney U test) (**Figure 1D**). Pearson correlation of LDH levels with AHN-related parameters revealed modest correlations with the absolute numbers of leukocytes (r=0.37, *P*<0.001) (**Figure 3A**). Within AdvSM, patients with LDH levels ≥260U/L (n=68), the threshold of ≥ or <400U/L significantly impacted the OS in this cohort (0.9 vs. 1.9 years, *P*=0.039, log-rank test) (**Figure 3C**).

Further risk stratification in IPSM-AdvSM1/2 patients. Risk stratification was performed by using the previously published International Prognostic Scoring System for Mastocytosis (IPSM)[11], a multiparametric (age ≥/<60, tryptase, ≥/<125ng/mL, leukocytes ≥/<16/nL, hemoglobin ≥/<11g/dL, platelets ≥/<100/nL, with one point for each of these parameters and minus one point for skin involvement resulting in a three tired risk model consisting of a total score between -1 and 5 points) scoring system. In 55 AdvSM patients with an IPSM risk score of IPSM-AdvSM1/2 (i.e -1 to 1 IPSM score points), assignment of 1 point to each of 5 serum parameters exceeding the described cut-off values (serum tryptase, alkaline phosphatase, ß2-microglobulin, LDH, albumin; **Table 1**) allowed a further risk stratification with a median OS of low- (0 points, n=9), vs. high-risk (2-4 points, n=46) patients of 9.9 vs. 3.6 years, respectively (*P*=0.027, log-rank test) (**Figure 2C**).

Complementary GREM-based analyses

AP isoenzymes. AP isoenzymes (liver, gall bladder, bowel, BM) were measured in 24 AdvSM patients. Compared to standard values, increased AP isoenzymes were

predominantly derived from liver and gall bladder (**Figure 4A**). The most robust, yet still moderate, correlations were seen with other liver parameters (gamma-GT, r=0.67, *P*<0.001, albumin, r=-0.37, *P*<0.001, INR, r=-0.49, *P*<0.001, all Pearson correlation) and the (relatively) poor correlation with bone and BM parameters (BM mast cell infiltration, r=0.35, *P*<0.001, Pearson correlation; osteoporosis, η=0.09, *P*<0.001 osteolysis, η=0.16, *P*<0.001, both Spearman correlation) further substantiated the strong association between elevated AP and liver involvement/damage (**Figure 4B**). AP levels ≥150U/L (optimal cut-off assessed per ROC analysis) significantly impacted on OS (10.5 years vs. 2.9 years, *P*<0.001, log-rank test) (**Figure 4C**).

Vitamin B12. Median vitamin B12 levels (n=334) were significantly higher in AdvSM compared to ISM (1,275U/L vs. 401U/L, *P*<0.001, Mann-Whitney U test) or SSM (353U/L, *P*<0.001, Mann-Whitney U test) (**Figure 5B**). Elevated vitamin B12 levels correlated with markers of advanced disease (e.g. high *KIT* D816V allele burden, r=0.45, *P*<0.001; elevated AP, r=0.51, *P*<0.001, both Pearson correlation) (**Figure 5A**). The area under the curve for predicting AdvSM was 0.795 (95% confidence interval [CI] 0.744-0.847, *P*<0.001, ROC analysis) and the optimal cut-off value was 400U/L with a sensitivity of 82% (Youden index 0.307) (**Figure 5B**). In AdvSM, vitamin B12 levels ≥2,000U/L conferred into worse outcome (median OS 4.1 vs. 2.6 years, *P*=0.037, log-rank test) (**Figure 5D**).

C-reactive protein. The median CRP levels (n=405) were markedly increased in AdvSM patients compared to either ISM (10.0 mg/dL vs. 2.9 mg/dL, *P*<0.001, Mann-Whitney U test) or SSM (3.7mg/dL, *P*<0.001, Mann-Whitney U test). Within AdvSM subvariants, the highest levels were found in SM-AHN (11 mg/dL) vs. ASM (3.5

mg/dL, *P*=0.057) or MCL (7.6 mg/dL, *P*=0.008, Mann-Whitney U test). Concomitant infections or autoimmune diseases as confounding conditions could be excluded in 390/405 (96%) patients.

Creatinine-adjusted β2-microglobulin. Elevated ß2-microglobulin levels were strongly associated with poor OS. Median OS was similarly impacted in AdvSM patients with β2-microglobulin levels ≥2.5mg/L independently whether or not the levels were adjusted for sex and age-matched serum creatinine values >ULN (not adjusted: median OS was 4.1 years vs. not reached, *P*<0.001; median OS 3.9 years vs. not reached, *P*=0.010, log-rank test).

DISCUSSION

Diagnosis and prognostication of ISM and AdvSM warrants application of comprehensive clinical (e.g. organomegaly, weight loss), morphological (e.g. BM mast cell infiltration, blood counts), serological (e.g. tryptase, AP) and molecular studies (e.g. *KIT* D816V, additional somatic mutations).[5,6,13,15,22-28] Due to the frequent absence of skin involvement and of typical MC mediator-related symptoms in AdvSM, many patients are misdiagnosed or the diagnosis may be significantly delayed,[15,24] unless disease-specific features and markers, such as BM MC infiltration, serum tryptase, or *KIT* D816V are considered and comprehensively studied. Although often used as a serological marker for screening of SM, an elevated serum tryptase may also be detected in patients with kidney failure, hereditary alpha tryptasemia, obesity, and other myeloid neoplasms.[16,29-33] While there is a certain correlation between serum tryptase levels and the subtype of SM, the differentiation between ISM, SSM or AdvSM is not straightforward. Therefore,

additional serologic parameters have been analyzed in the context of SM. One example is the AP which is typically elevated in patients with AdvSM with liver involvement. However, only a few comprehensive studies have systematically analyzed multiple serum chemistry parameters in patients with SM. Based on two robust patient registries, the ECNM registry and the GREM registry, we here show that a thorough and careful investigation and interpretation of certain serum chemistry parameters substantially supports the clinician in establishing the correct diagnosis, subclassification and prognostication of patients with SM.

While serum tryptase levels were highest in MCL, a level below 125µg/L in indolent phase disease was clearly associated with a more favorable outcome in ISM, BMM and SSM patients thus verifying the established cut-off level for differentiation between ISM and BMM.[34] In addition to serum tryptase, most valuable serum parameters include an elevated AP and hypoalbuminemia, which are widely included as C-findings in diagnostic criteria, recently established prognostic scoring systems and response criteria.[10,11,35] Based on AP isoform analyses, increased levels should be interpreted as liver involvement/damage by MC infiltration rather than as derived from bone involvement, e.g. indicating osteosclerosis or osteolyses. An elevated LDH correlated moderately with leukocytosis, monocytosis and eosinophilia indicating presence of an AHN[36]. The careful cross-assessment of serum tryptase representing the MC component, LDH (plus monocytes/eosinophils) representing the AHN component and the *KIT* D816V variant allele frequency potentially indicative for both components may therefore allow a rapid and thorough interpretation regarding the contribution of SM or AHN to relevant organ damage such as cytopenias and clinical parameters of liver dysfunction. It offers a first estimation of a sensible use of KIT targeted treatment.[4,13]

Elevated vitamin B12 is a known, unspecific finding in various (myeloid) neoplasms.[37-39] ROC analyses revealed that a cut-off level of ≥400U/L (in the absence of supplementation) is indicative of AdvSM and that a level ≥2,000U/L correlated with adverse prognosis allowing its complimentary use for assessment of response and progression. Significant weight loss may result from malabsorption and malnutrition but also from chronic inflammation, and it was regularly accompanied by hypoalbuminemia and elevated CRP with median CRP values being higher in AdvSM as compared to indolent SM (GREM cohort). Of interest, disease-associated symptoms such as night sweats or weight loss as well as the aforementioned serum chemistry parameters responded significantly on targeted treatment with midostaurin or avapritinib in most patients.[40-43]

Several prognostic scoring systems have recently been established for patients with SM and its subtypes. Whereas some scores are based on clinical parameters with inclusion of serum chemistry parameters, e.g. tryptase (IPSM), AP (GPSM) and β2-microglobulin (GPSM), and the WHO classification, others are based on additional molecular abnormalities. By now, no serum chemistry-specific score has been developed. In the present study, assignment of 1 point each to 5 individual serum parameters based on ROC analyses defined cut-off values for tryptase, AP, ß2-microglobulin, LDH and albumin allowed to create a serum score with differentiation between low-, intermediate and high-risk SM patients (median OS 15.1 vs. 3.4 vs. 1.5 years, P<0.001). Further adjunction of serum markers in IPSM AdvSM 1/2-weighted patients identified patients at higher risk in these otherwise favorable AdvSM subgroups. However, given the limited power of the log-rank test, a possible overestimation of the observed effect size could not be excluded with complete

certainity, necessitating verification in a larger, higher-powered analysis. Unfortunately, information on ß2-microglobulin was missing in 78% of patients from the ECNM cohort. We therefore strongly recommend the routine assessment of β2-microglubulin in the diagnostic work-up of SM.

Given the unavailability of molecular analyses for non-hematologists and the multidisciplinary management of SM patients by dermatologists, gastroenterologists and immunologists/endocrinologists, it is of substantial relevance to identify easily available red flags pointing out on a more thorough diagnostic work-up, e.g. bone marrow biopsy and molecular analyses, and the potential need for more aggressive treatment.

We conclude that beside its value for diagnosis according to WHO/ICC (albumin, tryptase, AP) classifications and various risk scoring systems (β2-microglobulin, AP), routine serum chemistry should also include vitamin B12, LDH and CRP, individually contributing to improved diagnosis, subclassification and prognostication. Those easily accessible parameters combine advantages regarding availability, reliability and observer-objectivity and may therefore serve as feasible tools in the clinical practice of medical doctors from all specialties involved in the management of SM patients.

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AUTHORSHIP CONTRIBUTIONS

Conception and design: JL, WRS, PV, AR, JS

Provision of study materials or patients: All authors

Collection and assembly of data: All authors

Data analysis and interpretation: JL, AR, JS

Manuscript writing: All authors

Final approval of manuscript: All authors

Accountable for all aspects of the work: All authors

CONFLICT OF INTEREST DISCLOSURES

	Within this study	Outside this study
Johannes Lübke	None	None
Alicia Schmid	None	None
Deborah Christen	None	Advisory Board and Honoraria: Blueprint Medicines
Hanneke Oude Elberink	None	Advisory Board and Honoraria: Blueprint Medicines
Bart Span	None	None
Marek Niedoszytko	None	None
Aleksandra Gorska	None	None
Magdalena Lange	None	None
Karoline V. Gleixner	None	None
Emir Hadzijusufovic	None	None
Oleksii Solomianyi	None	None
Irena Angelova-Fischer	None	None

Roberta Zanotti	None	Advisory Board and Honoraria: Blueprint Medicines, Novartis, Cogent Biosciences
Massimiliano Bonifacio	None	None
Patrizia Bonadonna	None	Advisory Board and Honoraria: Blueprint Medicines, Novartis
Khalid Shoumariyeh	None	Advisory Board and Honoraria: Blueprint Medicines, Novartis; Member of the Study Sterring Committee (SSC) for the CGT9486 Study of Cogent Biosciences
Nikolas von Bubnoff	None	None
Sabine Müller	None	None
Cecelia Perkins	None	None
Chiara Elena	None	Advisory Board and Honoraria: Blueprint Medicines, Gilead
Luca Malcovati	None	None
Hans Hagglund	None	None
Mattias Mattsson	None	None
Roberta Parente	None	None
Judit Varkonyi	None	None
Anna Belloni Fortina	None	None
Francesca Caroppo	None	None
Alexander Zink	None	None
Knut Brockow	None	Advisory board and honoraria: Blueprint Medicines, Novartis
Christine Breynaert	None	None
Dominique Bullens	None	None
Akif Selim Yavuz	None	None
Michael Doubek	None	None
Vito Sabato	None	Advisory Board: Blueprint Medicines, Novartis
Tanja Schug	None	None
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582 **TABLES**

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Table 1. Univariate and multivariable analysis regarding overall survival of 685 patients with systemic mastocytosis enrolled within the ECNM.

	Univariate		***Multivariable***	
Characteristics	*HR (95% CI)*	*P*	*HR (95% CI)*	*P*
Tryptase (n=625) ≥/<125µg/L	3.4 (2.6-4.4)	<0.001	1.8 (0.9-3.6)	0.078
Alkaline phosphatase (n=565) ≥/<150U/L	3.5 (2.7-4.4)	<0.001	1.4 (0.7-3.0)	0.334
ß2-microglobulin (n=158) ≥/<2.5mg/L	4.8 (2.9-7.9)	<0.001	2.7 (1.4-5.4)	0.004
Lactate dehydrogenase (n=533) ≥/<260U/L	2.0 (1.5-2.7)	<0.001	2.1 (1.1-4.0)	0.018
Albumin (n=508) </≥34g/dL	3.7 (2.7-4.9)	<0.001	3.3 (1.7-6.5)	0.001

Abbreviation: HR, hazard ratio
Analysis included variables with ROC-assessed cut-offs.

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Table 2. 1,951 patients with systemic mastocytosis (ECNM) stratified according to LDH ≥/<260U/L.

	LDH ≥260U/L	LDH <260U/L	*P*
Number of patients at diagnosis, n (%)	236 (12)	1715 (88)	
Age in years; median (range)	55 (18-90)	50 (5-87)	<0.001
Male, n (%)	149 (63)	789 (46)	<0.001
C-Findings			
Hemoglobin, g/dL; median (range)	13 (4-18)	14 (4-18)	<0.001
<10g/dL, n (%)	44 (19)	107 (6)	<0.001
Platelets, x10^9/L; median (range)	227 (0-958)	251 (5-893)	NS
<100x10^9/L, n (%)	51 (22)	141 (8)	<0.001
ANC, x10^9/L; median (range)	4 (0-9)	4 (0-76)	<0.001
<1x10^9/L, n (%)	7 (3)	20 [3]	0.025
Alkaline phosphatase, U/L; median (range)	84 (34-1696)	77 (20-1407)	0.001
>150U/L, n (%)	58 (27)	206 (13)	<0.001
Albumin level, g/L; median (range)	41 (16-57)	44 (20-57)	<0.001
<34g/L, n (%)	26 (14)	58 (4)	<0.001
Ascites, n (%)	23 (10)	97 (6)	0.020
Portal hypertension, n (%)	7 (3)	42 (3)	NS
Weight loss (>10 % over last 6 months), n (%)	44 (19)	172 (10)	<0.001
Osteolytic lesions, n (%)	7 (3)	47 (3)	NS
B-Findings			
Dysmyelopoiesis, n (%)	64 (28)	206 (13)	<0.001
BM MC infiltration, %; median (range)	15 (1-90)	10 (0-100)	NS
Serum tryptase level, µg/L; median (range)	43 (1-4530)	36 (2-4980)	NS
>125µg/L, n (%)	59 (27)	330 (20)	0.020
Splenomegaly, n (%)	65 (28)	291 (17)	<0.001
Hepatomegaly, n (%)	58 (234)	249 (1662)	<0.001
Lymphadenopathy, n (%)	32 (210)	148 (10)	0.009
Other relevant findings			
Leukocytes, x10^9/L; median (range)	7.7 (0.6-129.3)	6.7 (1.0-97.3)	<0.001
>16.000x10^9/L, n (%)	47 (20)	55 (3)	<0.001
Monocytes, x10^9/L; median (range)	0.5 (0.0-18.7)	0.4 (0.0-8.2)	<0.001
>0.8x10^9/L	44 (58)	16 (12)	<0.001
Eosinophils, x10^9/L; median (range)	0.2 (0.0-35.0)	0.1 (0.0-18.5)	0.006
>1.5x10^9/L	17 (23)	6 (4)	<0.001
KIT D816V positive, n (%)	163 (82)	1308 (88)	0.029
BM MC burden in smears, %; median (range)	5 (0-88)	2 (0-100)	0.041
Outcome			
Follow-up, years, median (range)	1.34 (0.0-22.3)	2.1 (0.0-28.6)	NS
Death, n (%)	65 (30)	177 (14)	<0.001

Abbreviations: ANC, absolute neutrophil count; AHN, associated hematologic neoplasm; BM, bone marrow; GI, gastrointestinal; MC, mast cell; NS., not significant
For subgroup analyses (presence versus absence of AHN) in patients with an LDH ≥260 U/L and <260 U/L, please see **Online Repository Table E3**. The *P* values refer to the Mann-Whitney U test or the Fisher's exact test.

FIGURE LEGENDS

Figure 1 (ECNM). Violin plots on the distribution of various serum chemistry markers throughout the systemic mastocytosis subtypes. The *P* values refer to the Mann-Whitney U test. Abbreviations: AdvSM, advanced systemic mastocytosis; ASM, aggressive systemic mastocytosis; ISM, indolent systemic mastocytosis; MCL, mast cell leukemia; SM-AHN, systemic mastocytosis with an associated hematologic neoplasm; SSM, smoldering systemic mastocytosis.

Figure 2 (ECNM). (A) Kaplan-Meier estimates of overall survival (OS) in SM patients with 0 points, 1-2 points versus 3-5 points by assignment of 1 point each to serum tryptase ≥125µg/L, AP ≥150U/L, LDH ≥260U/L, albumin ≤34mg/dL and ß2-microglobulin ≥2.5mg/L; [a]power = 1.000, [b]power = 1.000. (B) Kaplan-Meier estimates of overall survival (OS) in SM patients based on the multivariable model with 0 points, 1.5 points versus ≥1.5 points by assignment of 1 point to LDH ≥260U/L, and 1.5 points each to albumin ≤34mg/dL and ß2-microglobulin ≥2.5mg/L; [c]power = 1.000, [d]power = 0.998. (C) Kaplan-Meier estimates of OS in IPSM-AdvSM1/2-weighted patients with 0 points versus ≥1 points. [e]power = 0.441. The *P* values refer to the log-rank test.

Figure 3 (ECNM). (A) Pearson correlation of the lactate dehydrogenase (LDH) levels with other disease specific parameters (reflecting the systemic mastocytosis and the associated hematologic neoplasm component). *Significant *P* values adjusted by Holm-Bonferroni. (B) Kaplan-Meier estimates of overall survival in SM patients stratified according to LDH levels (260-400U/L vs. ≥400U/L); [a]power = 0.995. (C) Kaplan-Meier estimates of overall survival in AdvSM patients according to LDH levels (260-400U/L vs. ≥400U/L); [b]power = 0.720. The *P* values refer to the log-rank test.

Abbreviations: AdvSM, advanced systemic mastocytosis; ANC, absolute neutrophil count; AP, alkaline phosphatase; β2MG, ß2-microglobulin; Eos, eosinophil count; Hb, hemoglobin; LDH, lactate dehydrogenase; MC-I, mast cell infiltration; KIT, *KIT* D816V allele burden; Monos, monocyte count; Plt, platelets; SM, systemic mastocytosis; WBC, white blood cells.

Figure 4 (GREM). (A) Violin plots on the distribution of the alkaline phosphatase (AP) subtypes in 24 advanced systemic mastocytosis (AdvSM) patients. (B) Pearson and Spearman correlation of the AP levels with other (liver and bone marrow) specific parameters. *Significant *P* values adjusted by Holm-Bonferroni. (C) Kaplan-Meier estimates of overall survival in AdvSM patients stratified according to AP levels ≥/<150U/L. [a]power = 0.974. Abbreviations: AdvSM, advanced systemic mastocytosis; Alb, albumin; AP, alkaline phosphatase; ASAT, aspartate aminotransferase; Bili, bilirubin; MC-I, BM mast cell infiltration; O.poris, osteoporosis; O.lysis, osteolysis.

Figure 5 (GREM). (A) Pearson correlation of the vitamin B12 levels with other disease specific parameters. *Significant *P* values adjusted by Holm-Bonferroni. (B) Receiver operating characteristic curve for optimal cut-off assessment regarding diagnosis of ISM vs. AdvSM. (C) Kaplan-Meier estimates of overall survival (OS) in SM patients according to vitamin B12 levels (<800U/L vs. ≥800-1800U/L vs. ≥1800U/L); [a]power = 0.948, [b]power = 0.627. (D) Kaplan-Meier estimates of OS in AdvSM patients (≥2000U/L vs. <2000U/L); [c]power = 0.586. The *P* values refer to the log-rank test. Abbreviations: AdvSM, advanced systemic mastocytosis; ANC, absolute neutrophil count; AP, alkaline phosphatase; ASM, aggressive systemic mastocytosis; β2MG, ß2-microglobulin; Eos, eosinophil count; Hb, hemoglobin; ISM, indolent systemic mastocytosis; LDH, lactate dehydrogenase; MC-I, mast cell

637 infiltration; MCL, mast cell leukemia; KIT, *KIT* D816V allele burden; Monos, monocyte
638 count; Plt, platelets; SM, systemic mastocytosis; SM-AHN, systemic mastocytosis
639 with an associated hematologic neoplasm; SSM, smoldering systemic mastocytosis;
640 WBC, white blood cells.

Figure 1

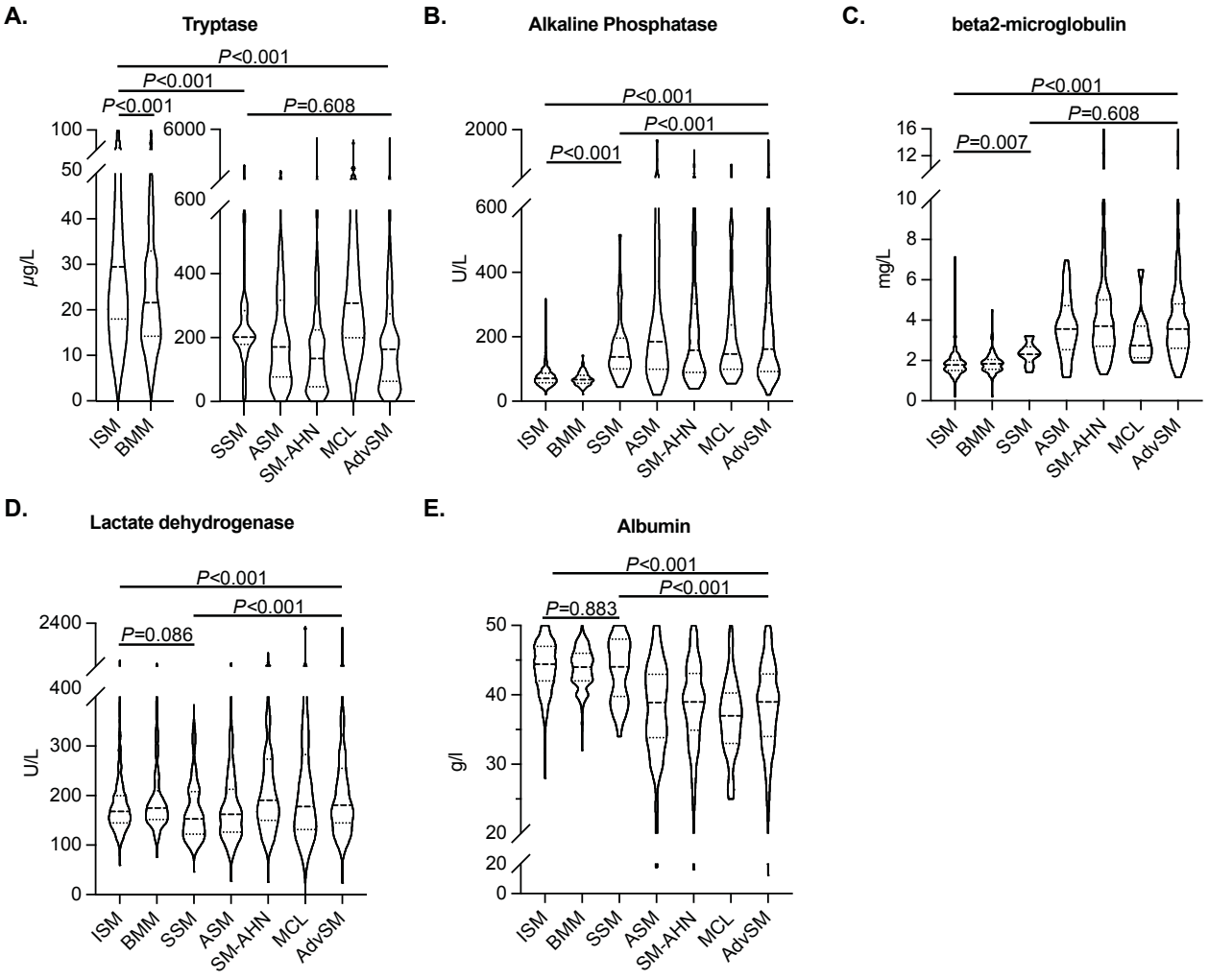
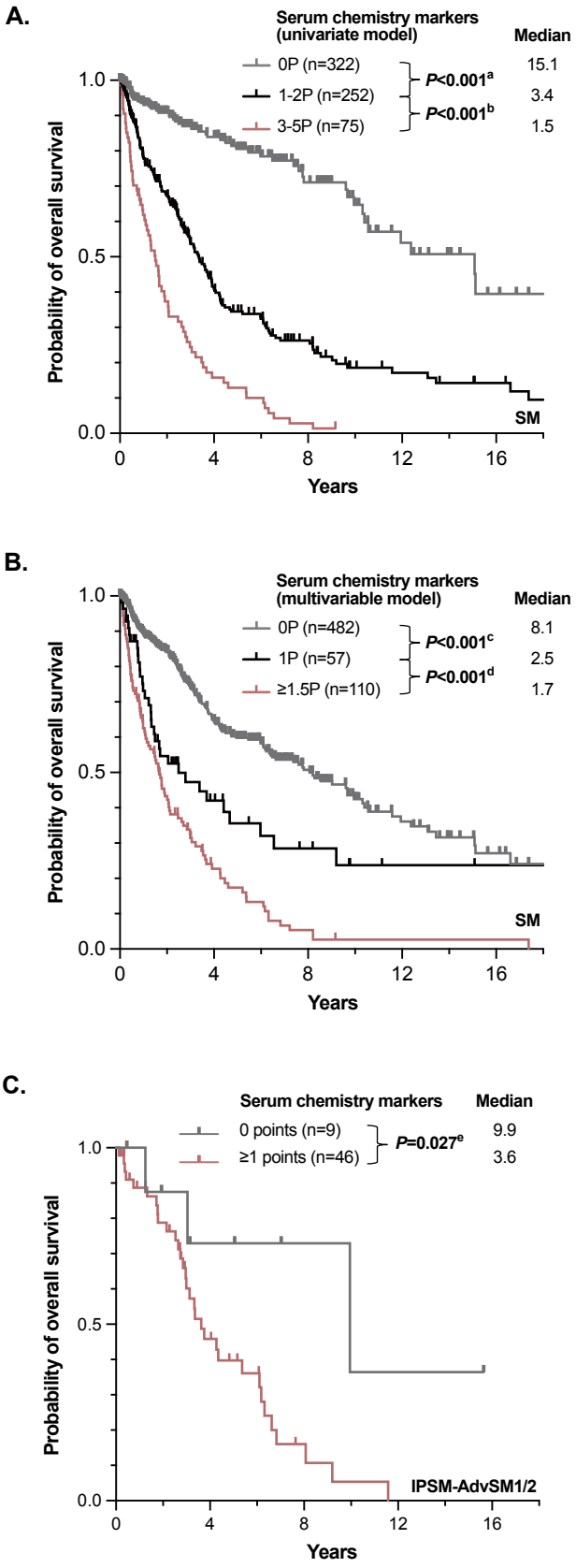


Figure 2



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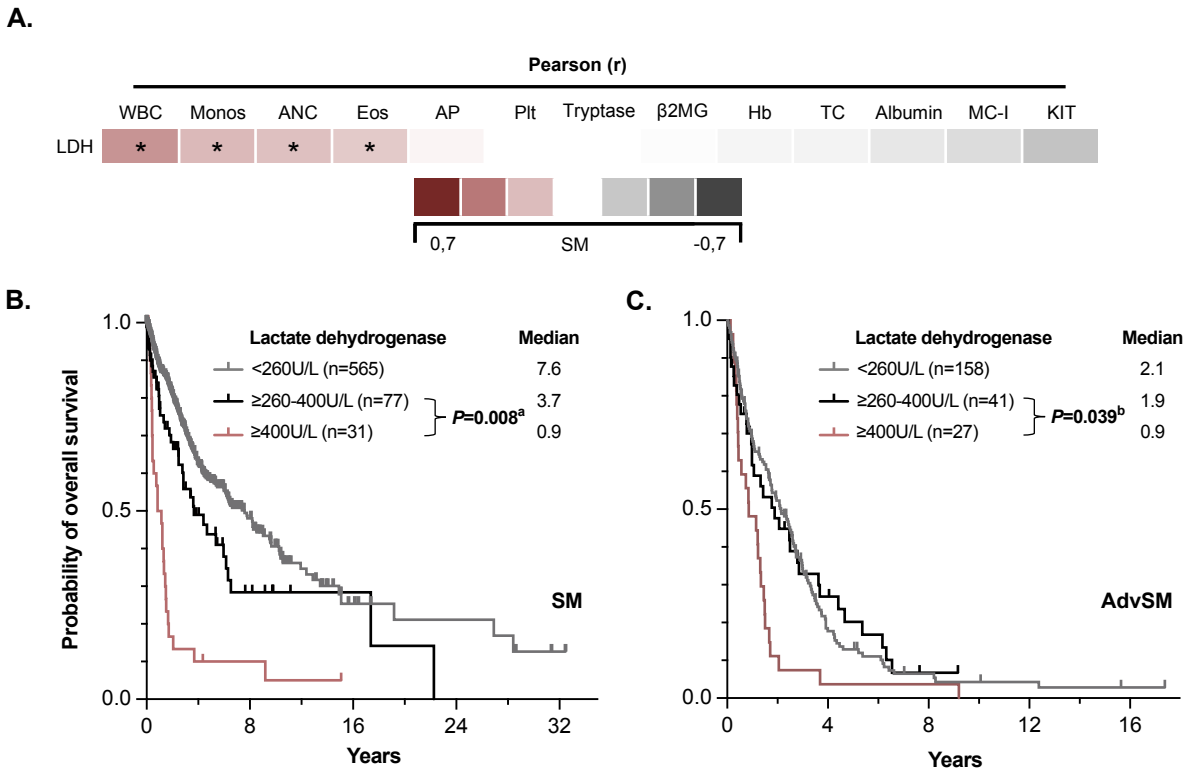

Figure 4

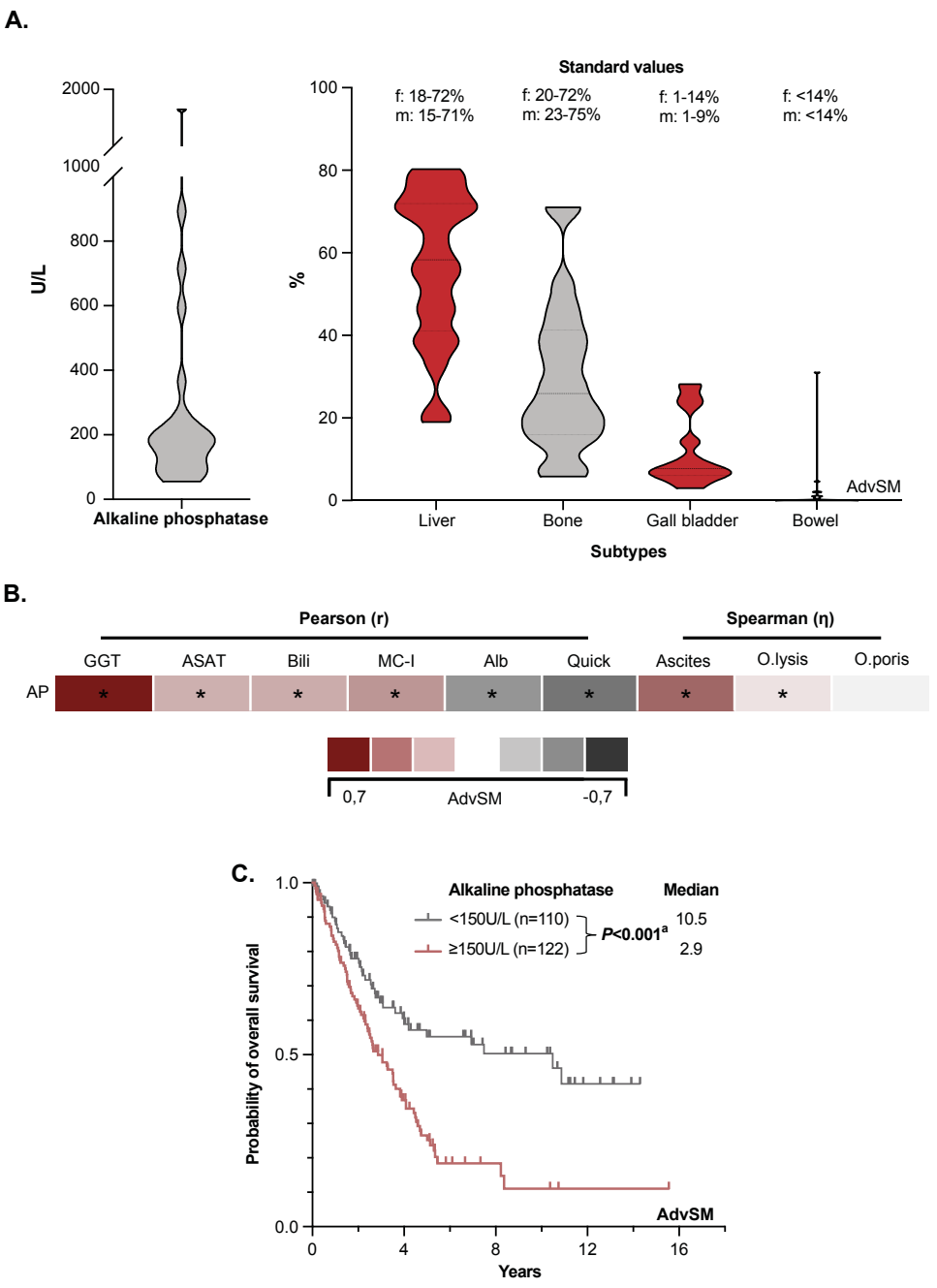

Figure 5