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Chapter 9

Manuscript in preperation

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Nederlands Mastocytose Centrum Groningen

SOLUBLE CD30 REFLECTS MAST CELL LOAD AND HYMENOPTERA ANAPHYLAXIS RISK IN MASTOCYTOSIS

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Abstract

Background: Mast cell CD30 expression has previously been found to be preferentially expressed in advanced forms of systemic mastocytosis (SM) compared to indolent systemic mastocytosis (ISM). CD30 is a trans-membrane receptor that is proteolytically cleaved, producing soluble CD30 (sCD30). Interaction between CD30 and its ligand on lymphocytes is critical for the development of allergic responses such as Hymenoptera venom anaphylaxis (HVAn) and can be inhibited by sCD30.

Objective: This study aims to uncover the relationship between sCD30, the classification of SM and the risk of HVAn.

Methods: Levels of sCD30 were measured in peri-diagnostic serum samples from 193 SM patients and 26 control samples.

Results: Serum levels of sCD30 were elevated in SM patients when compared to control patients, correlated with bone marrow mast cell infiltration (R= 0.264, P= 0.001) and were significantly higher in patients with advanced SM (median 136.3 ng/ml) compared to ISM patients (median 31.3 ng/ml). However, there was considerable overlap between disease categories and sCD30 did not predict survival in advanced SM. In a subgroup analysis of 118 stung ISM patients sCD30 operated as an independent predictor for a lower risk of HVAn (odds ratio: 0.728; P=0.016), together with mast cell load and age at latest sting. Moreover, sCD30 levels were inversely correlated to wasp specific IgE (R: -0.310; P=0.002), hinting at a possible desensitizing effect of mast cell derived sCD30.

Conclusion: Serum sCD30 levels pertain to mast cell load and the risk of HVAn, but not the classification of SM.

Introduction

Systemic mastocytosis (SM) is characterized by the clonal proliferation of abnormal mast cell in at least one extra-cutaneous tissue. SM is classified by WHO criteria following the level of tissue invasion and organ dysfunction.[1] The indolent form of systemic mastocytosis (ISM) is the most prevalent category of SM.[2] Patients suffering from ISM and its subcategory, smoldering systemic mastocytosis (SSM), have a near-normal life expectancy.[3] Mast cell mediator release symptoms, such as the frequent life threatening anaphylactic reactions to wasp and bee stings,[4] represent most of the disease burden in these patients.[5] Recent publications reveal that Hymenoptera venom anaphylaxis (HVAn) is most frequent in ISM patients with low mast cell burden. [6, 7] The advanced categories of SM, namely aggressive systemic mastocytosis (ASM), SM with an associated hematologic non-mast cell lineage disorder (SM-AHNMD) and mast cell leukemia (MCL), are hallmarked by aggressive tissue invasion and subsequent organ dysfunction.[1] Even with cytoreductive management, patients have a poor prognosis with a median survival of 2 to 44 months depending on the classification.[1, 2]

Biological markers used for diagnosing SM are the presence of the D816V *C-KIT* mutation, aberrant expression of CD25 and CD2 on mast cells and elevated levels of baseline serum tryptase or urinary histamine metabolites.[8] However, these markers fail to discriminate between indolent and aggressive forms of SM. Currently, the diagnosis of advanced SM is based on B and C findings, the former being clinical evidence of severe mast cell organ infiltration such as organomegaly, the latter evidence of organ dysfunction, such as cytopenia or ascites.[1] Initial reports indicated that aberrant immunohistochemical expression of CD30 by mast cells is preferentially observed in advanced categories of SM, making it a useful tool for grading SM, and speculates on a pathogenic and prognostic role for CD30.[9] These results were later confirmed using flow cytometry. In contrast, others have found CD30 to be broadly expressed in indolent and well differentiated forms of SM that lack expression of CD25. [10,11]

CD30 is a trans-membrane receptor limited in expression under physiological conditions to sub-populations of activated T and B cells.[12] Interaction between CD30 and its ligand CD30L on B and T lymphocytes is implicated in regulating memory antibody responses, Ig class switching to IgE and IgG,[13-15] and plays a critical role in the regulation of specific IgE (sIgE) and sIgE mediated diseases.[16, 17] Expression of CD30 by mast cells could interfere with physiological CD30- CD30L signaling, reducing sensitization rates and thereby causing the observed reduction of HVAn risk in ISM patients with a high mast cell load.[6]

CD30 is also a hallmark of hematological malignancies such as classical Hodgkin lymphoma and anaplastic large-cell lymphoma, where CD30 - CD30L signaling stimulates proliferation or apoptosis depending on the cell line under investigation.[18] CD30 positive (CD30+) cells shed a soluble form of CD30 (sCD30) after proteolytic cleavage of their extracellular domain. Serum sCD30 measurement using ELISA techniques has been successfully used to measure the tumor load of CD30+ neoplasms, correlating with treatment resistance and disease-free survival in classical Hodgkin lymphoma.[19, 20,21] Recently, sCD30 was found to be elevated in a small (n=37) cohort of mastocytosis patients compared to controls, however no relation could be found with the traditional marker of mast cell load, tryptase.[49] The aim of the present study is to investigate if sCD30 levels can differentiate between advanced and indolent SM patients, and if high sCD30 levels are associated with a reduction in HVAn risk.

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Methods

Patients

All consecutive adult patients admitted to the University Medical Center Groningen diagnosed with SM between 01-01-2004 and 01-09-2012 according to the World Health Organization (WHO) criteria were retrospectively assessed.[1, 8] This time period was chosen because of the large collection of archived serum samples available for the patients diagnosed at that time. Patients with a diagnosis of SSM, ASM, SM-ANHMD or MCL and available stored serum samples were stratified to the advanced systemic mastocytosis subset (AdvSM), whereas patients with a diagnosis of ISM and available stored serum samples taken within six months of diagnosis were stratified to the low-grade subset according to criteria previously established.[9] As an illustrative reference group, serum samples from 24 healthy and 2 atopic volunteers working at the department of Pathology were analysed.

Exclusion criteria

Patients that had undergone cytoreductive therapy prior to sampling were excluded. Patients with a positive history for active EBV infection, HIV infection, hepatitis B or C infection, rheumatoid arthritis, psoriasis, inflammatory bowel disease, Hashimoto's thyroiditis, Graves' disease, lupus erythematosus, primary biliary cirrhosis or a history of a non-mastocytosis CD30 expressing neoplasm were excluded in an attempt to minimize non mast cell derived sCD30.[18, 22] Even though atopic disorders are associated with elevated levels of sCD30,[23] the presence of atopic disorders was deliberately not chosen as an exclusion criterion as this is a minor risk factor for sensitisation to Hymenoptera venom.[24] The diagnosis of HVAn was based on international accepted clinical criteria. [25] A history of Hymenoptera stings was established from the patient charts, a questionnaire sent to the patients or telephone calls with individual patients.

Biochemical analysis

Duplicate sCD30 measurements were performed by enzyme immunoassays in two runs (ELISA; Bender MedSystems, Vienna, Austria) according to the manufacturer's instructions. Total and specific IgE to common wasp (ImmunoCAP, i3) venom and to the recombinant protein *Vespula* antigen 5 (rVes V 5, ImmunoCAP, i209, all Phadia) were measured using the fluor-enzyme-immunoassay Phadia Immunocap (Phadia; Uppsala, Sweden).

Basel serum tryptase (bsT) levels were determined using the B12 assay (ImmunoCAP Tryptase, Thermo Fisher Scientific, Uppsala, Sweden). Reference values for healthy individuals are those reported by Phadia, showing a geometric mean level of 3.8 µg/l and an upper 95th percentile of 11.4 µg/l. The inter-assay analytical coefficient of variation in our laboratory is 5.8%.

To measure methylhistamine (MH) and methylimidazole acetic acid (MIMA), urine samples were collected after an overnight fast, discarding the first voiding after wakening. During the 24h before urine collection, patients were asked to refrain from histamine-rich foods and drinks, such as sauerkraut, canned fish, yoghurt, and wine. MH and MIMA were determined by an isotope-dilution mass fragmentographic method as described previously. [26, 27] The percentage of mast cells in bone marrow aspirate was determined by flow-cytometric immunophenotyping of ≥300,000 events for $CD45^{low}$ and $CD117^{high}$ expression.

Statistical methods

Statistical analyses were performed with SPSS 20 software (SPSS, Chicago, IL, USA). Continuous parametric data are presented as mean ± SD, non-parametric continuous data are represented as median and interquartile range (IQR). Differences per patient group in characteristics and the parameters sCD30, bsT, MH and MIMA were compared using the Mann-Whitney U test, Student's t-Tests or the Pearson chi-square test as applicable.

A Cox proportional hazards regression model was used for univariate analysis of the relation between sCD30 and survival in AdvSM. The relation between sCD30 and HVAn was investigated in a multivariate logistic regression model based on previously identified predictors, mast cell load and age at latest sting, in 118 patients with known Hymenoptera venom exposure in adult life (age ≥15 years).[6] Model building consisted of conditional stepwise backward exclusion of variables in the multivariate analysis with a P value ≤0.25 in univariate analysis. The probability of P for stepwise removal was 0.10. Multicollinearity between predictors in the multivariate analysis was assessed using the variance inflation factor. A two-sided P value <0.05 was taken to indicate statistical significance for all tests. Correlations were assessed using the Pearson's and Spearman's correlation coefficients as appropriate. The Medical Ethical Review Board of the University Medical Center Groningen declared that the study has been performed in accordance with regulations of the review board for publication of patient data.

Results

Of the 260 patients diagnosed with SM in the UMCG Groningen between 01-01-2004 and 01-09-2012, 61 were excluded due to unavailable peri-diagnostic serum samples and an additional 6 patients were excluded due to a concomitant disease listed in the exclusion criteria, resulting in a total of 193 SM patients. All patient characteristics are displayed in Table I. The majority suffered from ISM (n= 169, 87.6%), the remaining 24 from SSM (n=4), ASM (n=7), SM-AHNMD (n=10) or MCL (n=3). The time between diagnosis and sampling of the sera used for sCD30 analysis did not differ significantly between AdvSM (median 0 months; IQR 0 – 8 months) and ISM (median 0 months; IQR= 0 - 1 month; P = 0.117) patients. Differences between AdvSM and ISM patients were found for age at sampling (P < 0.001), the presence of urticaria pigmentosa (P = 0.047) and mast cell load parameters bsT, MH and MIMA (P< 0.001).

Serum sCD30 and classification of SM

Levels of sCD30 per disease category are displayed in Figure 1. Serum levels of sCD30 were found to be almost twofold higher in SM patients (median 34.8 ng/ml; IQR 22.4 – 53.7 ng/ml) compared to the 26 controls (median 18.0 ng/ml; IQR 13.7 – 29.1 ng/ml, P<0.001) Similarly, serum levels of sCD30 levels were found to be more than fourfold higher in patients with AdvSM (median 136.3 ng/ml; IQR 57.5 – 311.3 ng/ml) compared to ISM patients (median 31.3 ng/ml; IQR 21.1 – 44.0 ng/ml, (P<0.001). Circulating sCD30 seems to be partly related to the category of SM and partially to the underlying mast cell burden: in the total group of 193 SM patients sCD30 levels were significantly correlated to mast cell load parameters bsT, MH and MIMA (R= 0.384 – 0.403, P<0.001, Figure 2) and the percentage of mast cells in bone marrow smears (R= 0.264, P= 0.001).These relations remain significant in the subgroup of 24 AdvSM patients.

However, no significant correlation was found between bone marrow mast cell infiltration and sCD30 in the ISM subgroup (Table II).

sCD30 and prognostication of advanced systemic mastocytosis patients.

The median follow- up duration for the 24 included AdvSM patients was 24.3 months, during which 13 deaths were noted. Univariate cox regression analysis revealed no significant relation between sCD30 and survival ($P = 0.206$, HR 1.003). Additionally, no correlation was found between sCD30 and the number of C findings (Table II).

Soluble CD30 and Hymenoptera venom anaphylaxis

The relationship between sCD30 and HVAn was investigated in a subgroup of 118 ISM patients with adult (age>15) Hymenoptera venom exposure. Fifty-four of these patients (45.8%) had developed HVAn, the majority following wasp stings (n = 50). Patients with a history of HVAn had significantly lower sCD30 levels (median 24.8; IQR 17.5 – 36.4) compared to patients without a history of HVAn (n=64; median 36.7; IQR 22.8 – 54.5; $P< 0.001$). Multivariate logistic regression analysis indicated that sCD30 is a significant independent predictor for a lower risk of HVAn, (OR 0.728; per 10 ng/mL increase; $P = 0.016$) after controlling for previously identified predictors mast cell load, as denoted by urinary MH, (OR 0.971; per 10 µmol/mol creat increase; $P= 0.017$) and age at latest sting (OR 1.058; per 1 year increase; $P= 0.001$). For the final predictors the variance inflation factor was < 1.09, indicating low multicollinearity. To summarize: in this model a 10 ng/mL higher serum sCD30 level predicted for a 14% lower absolute risk of HVAn independent of mast cell load and age. The results of the uni- and multivariate logistic regression analyses are displayed in Table 3.

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Table I Clinical and biochemical characteristics of the 193 included systemic mastocytosis patients.

		All patients					ISM patients with adult Hymenoptera venom exposure			
				Classification of SM				Hymenoptera venom anaphylaxis		
		Control	SM	ISM	AdvSM	P score		Yes	No	P score
Patients	n	26	193	169	24		118	54	64	
Age at sampling	years	29 ± 8.4	49.2 ± 13.2	47.6 ± 12.9	60.1 ± 10.7	<0.001	50.0 ± 11.4	52.1 ± 10.0	48.9 ± 12.0	0.125
Age at latest sting	years	-	41.4 ± 18.7	41.1 ± 18.9	-	-	45.7 ± 13.3	49.8 ± 10.7	42.0 ± 14.3	<0.001
Female	n, %	8 (30.8%)	112 (58.0%)	104 (60.9%)	8 (30.4%)	0.315	67 (56.8%)	29 (58.0%)	38 (55.9%)	0.914
ISM	n, %	-	169 (70.9%)	169 (70.9%)	(0%)	-	118 (100%)	54(100%)	61(100%)	-
SSM	n, %	-	4 (2.1 %)	0 (0.0%)	4 (17.4 %)	-				
ASM	n, %	-	7 (3.1 %)	0 (0.0%)	7 (26.1 %)	-				
MCL	n, %	-	3 (1.6 %)	0 (0.0%)	3 (13.0 %)	-				
SM-AHNMD	n, %	-	10 (5.2%)	0 (0.0%)	10 (43.5 %)	-				
HVAn	n, %	-	54 (29.9%)	54 (33.9%)	0 (0%)	<0.001	54 (45.8%)			
sCD30	ng/ml	18.0 (13.7 – 29.1)	34.8 (22.4 – 53.7)	31.3 (21.1 – 44.0)	136.3 (57.5 – 311.3)	<0.001	29.4 (20.4 – 42.9)	24.8 (17.5 – 36.4)	36.7 (22.8 – 54.5)	<0.001
BsT	µg/l	-	28.6 (14.0 - 51.2)	25.6 (16.8 - 44.4)	171.0 (96.3 - 292.0)	<0.001	26.7 (17.2 - 43.6)	23.9 (16.8 - 35.6)	30.7 (17.4 - 61.5)	0.107
MH	µm/mol cr.	-	296.5 (188.0 - 482.5)	265.0 (178.0 - 389.0)	1828 (986 – 3630)	<0.001	261.5(176.0 - 380.0)	230.5 (176.0 -339.0)	307.5 (178.0- 459.0)	0.039
MIMA	mm/mol cr.	-	3.4 (2.1 - 5.3)	3.1 (2.2 - 4.8)	27.3 (14.8 - 42.7)	<0.001	3.0 (2.1 - 4.5)	2.8 (2.1 - 3.7)	3.3 (2.2 - 5.9)	0.086
Follow up	Years	-	3.3 (0.9 - 6.9)	3.1 (1.6 - 5.5)	2.0 (1.4 - 3.8)	0.274	3.7 (1.4 - 6.9)	4.0 (1.7 - 7.1)	3.4 (1.4 - 6.7)	0.084
UP	n, %	-	141 (73.5 %)	129 (76.3 %)	12(50.0%)	0.047	83 (70.3%)	24 (44.4%)	59 (92.2%)	<0.001
BMI	kg/m²	-	25.5 (23.1 - 28.5)	25.6 (23.1 - 28.7)	23.9 (22.6 - 25.6)	0.189	26.1 (23.7 - 29.0)	25.4 (23.9 - 28.3)	26.7 (23.7 - 30.2)	0.440

ASM: aggressive systemic mastocytosis ;HVAn: Hymenoptera venom anaphylaxis; ISM: indolent systemic mastocytosis; MCL: Mast cell leukemia ; SM-AHNMD: systemic mastocytosis with an associated hematologic non-mast cell lineage disorder; SSM: smoldering systemic mastocytosis;

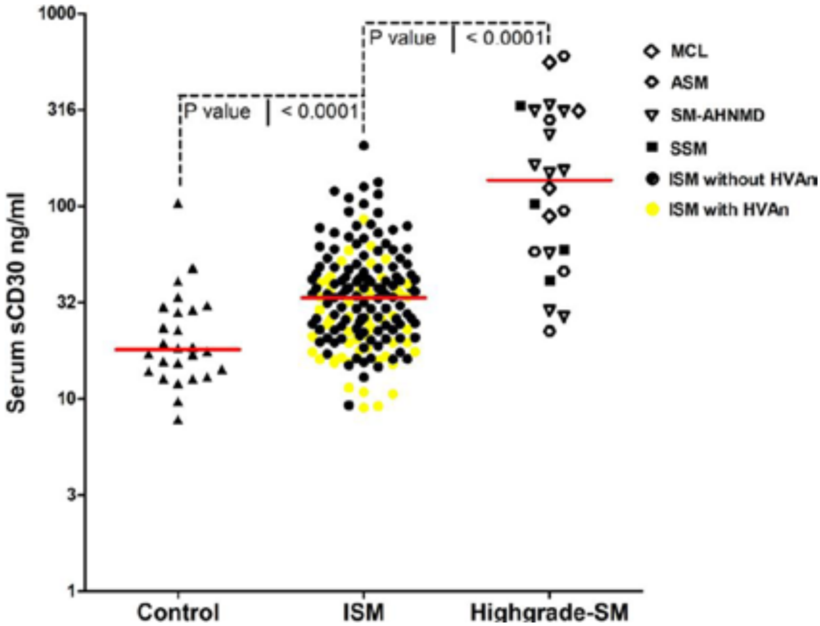
Table II Correlations between the biochemical and clinica parameters of systemic mastocytosis patients.

		bsT	MH	MIMA	%MC	C findings
All 193 SM patients						
sCD30	Spearman's R	0.385	0.384	0.403	0.264	
	P value	< 0.001	< 0.001	< 0.001	0.001	
24 AdvSM patients						
sCD30	Spearman's R	0.484	0.709	0.605	0.446	0.182
	P value	0.019	< 0.001	0.003	0.049	0.395
169 ISM patients						
sCD30	Spearman's R	0.218	0.204	0.233	0.331	-
	P value	0.004	0.008	0.002	0.713	-

Relationship with specific IgE

The relationship between sCD30 and sIgE was determined to elucidate a possible mechanism responsible for the protective association between sCD30 and HVAn. Wasps were the culprit insect in 88% of patient reported stings. Accordingly, the correlation between sCD30 and reactivity to Vespid sIgE was determined in the 104 ISM patients reporting adult (age ≥15 years) wasp stings. A significant inverse correlation was found between sCD30 levels and Vespid sIgE (Spearmans R: -0.310; P=0.002) and rVes V 5 (Spearmans R: -0.261; P=0.011). No correlation was found between sCD30 and total IgE (Spearmans R: 0.061; P=0.558). To investigate whether the inverse relation between sIgE and sCD30 could be a result of uptake and sequestration of sIgE by FceRI expressing mast cells, we studied the association between sIgE and the percentage of mast cells in bone marrow aspirate. Levels of Vespid sIgE and rVes V 5 did not correlate with mast cell numbers (Spearmans R: -0.118; P= 0.302 and Spearmans R: -0.029, P= 0.807 respectively).

Figure 1: Serum levels of soluble CD30 in 169 indolent systemic mastocytosis patients, 24 advanced systemic mastocytosis patients and 26 control patients.



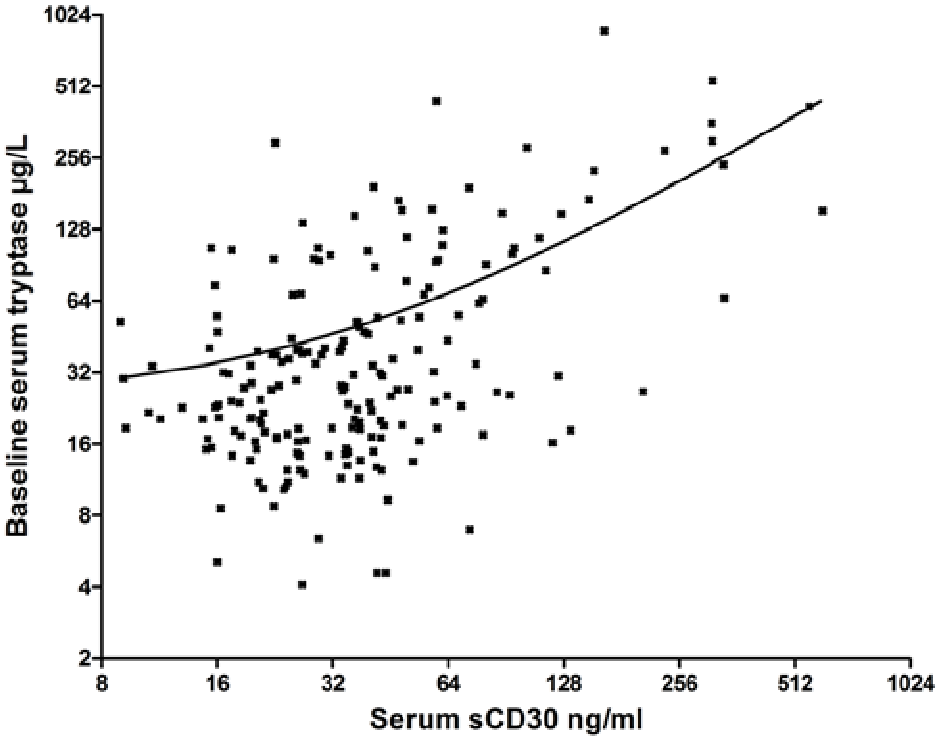
Data are given as mean ± SD, or median (IQR); AdvSM : advanced systemic mastocytosis; ASM: aggressive systemic mastocytosis; BMI: body mass index; BsT: base serum tryptase; Cr: creatinine; CM: cutaneous mastocytosis; HVAn: Hymenoptera venom anaphylaxis; ISM: indolent systemic mastocytosis; MCL: mast cell leukemia; MH: methylhistamine; MIMA: methylimidazole acetic acid; SM-AHNMD: systemic mastocytosis with an associated hematologic non-mast cell lineage disorder; SSM: smoldering systemic mastocytosis; UP: urticaria pigmentosa

Table III: Results of univariate and multivariate logistic regression analysis for predictors of Hymenoptera venom anaphylaxis in 118 indolent systemic mastocytosis patients with known adult Hymenoptera venom exposure.

	Univariate analysis		Multivariate analysis	
Predictor	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**
bsT (µg/l)[a]	0.885 (0.789 – 0.993)	0.038		[c]
MH (µmol/mol creat)[a]	0.972 (0.950 – 0.995)	0.015	0.971 (0.948 – 0.995)	0.017
MIMA (mmol/mol creat)[a]	0.164 (0.028 – 0.957)	0.045		[c]
sCD30 (ng/ml)[a]	0.690 (0.545– 0.874)	0.002	0.728 (0.562 – 0.943)	0.016
Age at most recent sting (years) [b]	1.048 (1.017 – 1.080)	0.002	1.058 (1.022 – 1.095)	0.001
Male gender [e]	1.082 (0.569 – 2.057)	0.809		[c]
Follow-up (years) [b, e]	1.037 (0.963 – 1.117)	0.331		[c]

OR: odds ratio; **CI**: confidence interval; **sCD30**: soluble CD30; **bsT**: basal serum tryptase; **MH**: methylhistamine; **MIMA**: methylimidazole acetic acid; **a** Odds ratio per 10 point increase in serum or urine concentrations **b** Odds ratio per 1 year increase **c** The variable was not selected during multivariate regression analysis. **e** The variable was not tested in multivariate regression analysis because of a P value > 0.25 in univariate regression analysis and no known clinical importance.

Figure 2: Correlation between serum levels of soluble CD30 and tryptase in 193 systemic mastocytosis patients



Discussion

This is the first study to our knowledge that investigated the relationship between sCD30 and HVAn in SM and we found sCD30 to be an independent predictor for a lower risk of HVAn. CD30 is an important co-stimulatory receptor supporting a Th2 polarized immune response.[29] In non-mastocytosis patients sCD30 is derived mainly from B- and T-lymphocytes and influenced by the amount of CD30 – CD30L signaling, events common in a Th2 polarized immune system.[30] Accordingly, serum sCD30 levels are elevated in a variety of allergic diseases, including Hymenoptera venom allergy.[31] In contrast, we found lower sCD30 levels in SM patients allergic to Hymenoptera venom, functioning as an independent predictor of HVAn risk. We hypothesized that the previously identified protective association between mast cell load and HVAn is the result of the sIgE suppressing qualities of mast cell expressed CD30.[23, 32, 33] However, the multivariate analysis reveals that mast cell load and sCD30 are both independent predictors of HVAn. Further research is needed to indentify by what mechanism a high mast cell load reduces the risk of HVAn The finding that sCD30 levels are lower in SM patients suffering from HVAn is striking and implicates a possible negative signaling pathway between mast cell derived sCD30 and B- and T-lymphocytes.

Human sCD30 is released after ligation of CD30, sCD30 itself promotes further proteolytic cleavage of CD30 and sCD30 acts as a decoy receptor by binding to CD30L with a high-affinity and blocking CD30 trans-membrane signaling.[6,34] Taken together this suggests that sCD30 acts as a negative feedback loop. The extra circulating mast cell derived sCD30 in SM could shift the balance of this negative feedback loop by interfering with constitutional CD30-CD30L interaction and thereby suppressing HVAn.

Investigations using murine CD30-CD30L knock out models and CD30L antagonistic antibodies comparable in function to sCD30 report a reduction of allergic rhinitis and asthma symptoms.[35] In addition, sCD30 binds B cell expressed CD30L, resulting in reverse signaling in the B-cell, [16, 17] which in turn further inhibits IgE antibody production and isotype switching.[36]

Suppression of sensitization in ISM patients by the antagonistic properties of sCD30 is further supported by the found inverse correlation between sCD30 and sIgE. These findings are consistent with reports of low sIgE in mice treated with antagonistic antibodies for CD30 and CD30L.[37, 38, 17, 39, 40] Others have speculated that the low levels of sIgE in SM patients are the result of the increased uptake and sequestration of circulating IgE by the increased abundance of high-affinity IgE receptor expressing mast cells.[30] Although this may partially explain the lower IgE levels in SM patients, murine investigations indicate that the prevalence of high affinity IgE receptors does not affect IgE levels.[41] Furthermore, in the current study no significant correlations were found between sCD30 and total IgE or between the percentage of mast cells in bone marrow aspirate and sIgE, suggesting that the observed inverse correlation between sCD30 and sIgE is specific for antigen-specific IgE only and not the result of increased binding of total IgE by mast cells. Suppression of sIgE levels by antagonistic binding of CD30L by sCD30 may be relevant for other malignancies as well; expression of CD30 is found in a variety of malignancies and IgE has been postulated as an important part of anti-tumor immune surveillance.[42, 43] Accordingly, CD30L expression has been found to be reduced in CD30 expressing malignancies.[18, 44] This raises the question whether aberrant expression of CD30 by mast cells is an immune escape mechanism working through suppression of sIgE.

Expression of CD30 and release of sCD30 could also directly influence the mast cells. In SM patients mast cells express both CD30 and its ligand CD30L, setting the stage for auto/paracrine signaling in the mast cell infiltrates. Reverse transmembrane signaling through mast cell expressed CD30L could account for mediator release symptoms by enhancing mast cell chemokine secretion.[45] The effects of CD30 transmembrane signaling on mast cells remains to be fully discovered, but the CD30 targeting antibody-drug conjugate brentuximab vedontin has been found to suppress IgE mediated degranulation.[49]

The results indicate that although sCD30 levels are significantly higher in AdvSM compared to low-grade SM, expression and release of sCD30 is not limited to AdvSM. Levels of sCD30 in ISM patients were significantly higher compared to the control group, albeit that both groups differed significantly in the age at sampling and gender, and correlated with mast cell load. Additionally, univariate survival analysis indicated no prognostic value for sCD30 and sCD30 levels did not correlate with C findings in AdvSM patients. These findings contradict earlier reports where immunohistochemical bone marrow mast cell CD30 expression was found to be limited mostly to AdvSM and suggested as a marker indicative of aggressive disease variants.[9] Mast cells as the origin for the elevated levels of sCD30 in ISM is supported by the recently published flow-cytometry data, illustrating that the identification of mast cell CD30 expression varies with the applied methodology. [10,28,11] Unfortunately, we were unable to immunohistochemically relate sCD30 levels to CD30 expression of mast cells in bone marrow to further substantiate the origin of the measured sCD30 levels. Pilot investigations revealed that the routine de-calcification step used in our laboratory using a mixture of 10% v/v acetic acid and 3.6% v/v formaldehyde rendered the archived bone marrow samples unfit for reliable immunohistochemical quantification of CD30 expression. Nevertheless, we feel that the strong correlations between sCD30, the mast cell load parameters bsT, MH, MIMA and the percentage of mast cells in bone marrow aspirate confirm that sCD30 levels in SM are mast cell dependent. However, sCD30 cannot substitute for BsT, MH or MIMA as a measurement of mast cell load or as a screening tool for systemic mastocytosis as it can be elevated in a large variety of diseases.

Importantly, this report is retrospective in nature and prospective research is needed before a true assessment of sCD30 as a risk factor for HVAn can be made. Prospective investigations are frustrated by the low incidence of mastocytosis, the infrequency of field stings, and the contra-indication of diagnostic sting challenges. However, during the course of this investigation the ISM patient with the lowest level of sCD30 (9.0 ng/ml) developed her first and regrettably fatal anaphylactic reaction following wasp stings, stressing the need of predictors identifying those at risk for HVAn.

In conclusion, serum sCD30 levels are elevated in all categories of SM and function as a parameter of mast cell load. Furthermore, sCD30 levels are inversely related to the risk of HVAn and Vespid venom specific IgE. We propose a model in which interference of B and T-cell CD30-CD30L signalling by mast cell expressed CD30 reduces the risk of HVAn in mastocytosis through suppression of specific IgE synthesis. These results implicate (s)CD30 as a potential prognostic and therapeutic target for hymenoptera venom allergy in ISM.

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