Journal of Molecular and Genetic Medicine

Vos, et al., J Mol Genet Med 2016, 10:1
DOI: 10.4172/1747-0862.1000201

Research Article Open Access

Differential Associations of MMP-2 and MMP-14 with Stromal Amounts and T-lymphocyte Presence in Ovarian Cancer

M Caroline Vos[1]*, Elise Bekers[2], Brenda L den Oudsten[3], Ella M. de Boed[2], Anneke A M van der Wurff[2], Toin H van Kuppevelt[4], Katarina Wolf[5] and Leon F A G Massuger[6]

[1]Department of Obstetrics and Gynaecology, ElisabethTweesteden Hospital, The Netherlands
[2]Department of Pathology, ElisabethTweesteden Hospital, The Netherlands
[3]Department of Medical and Clinical Psychology, Tilburg University, The Netherlands
[4]Department of Biochemistry, Nijmegen Centre for Molecular Life Sciences, Radboud University Medical Center, the Netherlands
[5]Department of Cell Biology, Radboud Institute for Molecular Life Sciences, The Netherlands
[6]Department of Obstetrics and Gynaecology, Radboud University Medical Center, The Netherlands

Abstract

Aim: Ovarian cancer prognosis is influenced by factors such as intratumoral T-lymphocyte presence and the amount of stroma formation relative to the epithelial tumor compartment. The regulation of these factors is unknown. Matrix metalloproteinases are involved in stromal remodeling and may be involved in T-cell trafficking as well. This study investigates the quantitative relationships between the matrix metalloproteinases MMP-2 and MMP-14 and both relative stroma amounts ('stroma percentage') and the presence of T lymphocytes in the stromal and epithelial compartments of ovarian cancer.

Patients and methods: In 86 patients with ovarian cancer, MMP-2 and MMP-14 expression and T-lymphocyte presence was determined using semi-quantitative immunohistochemistry. Stroma percentage was determined by area calculation in haematoxylin-eosin slides. Per slide, 3 random images at the tumor-stroma interface were investigated.

Results: Epithelial MMP-14 scoring correlated negatively with T-lymphocytes in tumor epithelium: correlation coefficient rho −0.36 ($p < 0.01$) for CD3, −0.30 ($p < 0.01$) for CD8 and −0.24 ($p < 0.05$) for CD45Ro. Stromal MMP-2 was positively related to stroma percentage (rho 0.29; $p < 0.01$). No significant correlations were found for MMP-14 with stromal amounts, or MMP-2 with T-cell presence in stroma or tumor epithelium.

Conclusion: Opposite to our hypothesis that MMPs reduce stromal amounts and permit T cell trafficking into both stromal and epithelial tumor parts, MMP expression was associated with higher relative stromal amounts and lower presence of T lymphocytes. In particular, we found that epithelial MMP-14 expression was negatively associated with T-lymphocyte presence in the tumor suggesting a role for MMP-14 in negatively regulating T-cell trafficking by other means. Stromal MMP-2 expression was associated with an increase in stromal amounts suggesting an ineffective feedback on tumor stroma volume growth by the expression of this gelatinase. More research is necessary to determine the specific actions of MMPs in ovarian cancer.

Keywords: Ovarian cancer; MMP-2; MMP-14; T-lymphocytes; Stroma percentage; Immunohistochemistry

Introduction

Ovarian cancer is known for its bad prognosis due to the lack of screening possibilities and the late onset of symptoms leading to diagnosis mostly in advanced disease. Despite intensive treatment with debulking surgery and chemotherapy, 5-year survival rates remain low [1].

In the past few years, the investigation of the stromal compartments of tumors has increasingly gained importance [2-5]. Stromal alterations are commonly found around the invasive front of a malignant tumor. One such alteration can be described as a desmoplastic stroma reaction, characterized by a dense fibrotic network with deposition of various types of fibrillar collagen. The presence of desmoplastic stroma correlates with adverse prognosis in for example endometrial cancer as well as in a subset of ovarian cancers [5-7].

The maintenance of stroma often correlates with the upregulation of matrix metalloproteinases (MMPs), especially MMP-14, as a feedback loop to keep stroma at constant density [8]. In cancer however, this process is often deregulated and connective tissues develop into dense tumor stroma despite MMP upregulation and the possibility to break up tissue again [9-11].

MMP's are ubiquitous zinc-dependent proteases that are important in numerous physiological and pathophysiological processes, including ovulation and reproduction but also cancer progression and metastasis [12]. Specifically, MMP-14 and MMP-2 are involved in physiological growth of ovarian follicles [13] and due to their high expression rate, could also be involved in the growth of malignant ovarian cysts [14,15]. Some studies in ovarian cancer have found that MMP-14 and MMP-2 are independent prognosticators, whereas others have concluded that their predictive value is limited [16-18].

Other important processes in a tumor's stromal compartment include the body's own defense to tumor growth by immunomodulation by different immune cell types, and include, i.e., cytotoxic T-cell-mediated tumor cell killing. These T-cells arrive from neighboring

***Corresponding author:** M. Caroline Vos, Department of Obstetrics and Gynaecology, ElisabethTweesteden Hospital, 5000 LC Tilburg, The Netherlands, Tel: 0031135392591; Fax: 0031135351513; E-mail: c.vos@elisabeth.nl

Received October 05, 2015; **Accepted** January 20, 2016; **Published** January 25, 2016

Citation: Vos MC, Bekers E, Oudsten den BL, de Boed EM, Wurff AAM, et al. (2016) Differential Associations of MMP-2 and MMP-14 with Stromal Amounts and T-lymphocyte Presence in Ovarian Cancer. J Mol Genet Med 10: 201 doi:10.4172/1747-0862.1000201

peripheral blood vessels and must travel through connective tissue compartments in order to reach and kill the tumor cells (Figure 1) [19].

The efficiency of T-cell trafficking is influenced by extracellular matrix (ECM) deposition, its geometry, which includes a guidance as well as a barrier function, and proteolytic remodeling which may open tracks of least resistance for moving cells. In malignant stroma the network densifies, develops thickened matrix fibers and thus may hinder T-lymphocyte migration [3].

In ovarian cancer, both the amount of stroma deposition relative to the tumor mass ("stroma percentage") and T-lymphocyte presence influence prognosis [19-21]. Both survival-promoting presence of tumour infiltrating T-lymphocytes and survival-impairing high stroma percentage is reported for advanced ovarian cancer [19-21]. However, whether and how these survival-determining factors correlate with modulated MMP-expression typical in ovarian cancers remains unknown.

We here explored in a large regional cohort with long-term follow-up whether MMP-2 and MMP-14 expression was associated with these stromal and stroma-derived processes in ovarian cancer for the reduction of stroma mass by ECM turnover and attraction of T-cell influx. Our data indicate, opposite to our expectations, that MMP-2 expression positively correlates with relative stromal amounts and MMP-14 negatively associates with T-cell presence in tumor tissue.

Patients and Methods

Clinical data

Included in this retrospective cohort were all 116 patients diagnosed with ovarian cancer between January 1997 and December 2003 at St. Elisabeth Hospital and Tweesteden Hospital, both in Tilburg, and at Amphia Hospital, Breda all in, the Netherlands. Patients who had undergone neo-adjuvant chemotherapy with secondary cytoreductive surgery were excluded (n = 11), because previous chemotherapy may have influenced the immunohistochemical results of MMP's and T-lymphocytes of histopathological material obtained at the time of surgery [22]. All patients were followed until March 2013. Four patients emigrated and were thus lost to follow-up. For 15 patients, no residual tumor material was available for complete immunohistochemistry, leaving 86 patients for which MMP-14 and MMP-2 expression and T-lymphocyte presence were determined. For a flowchart of the patients see Figure 2.

The patients underwent a laparotomy with staging or debulking

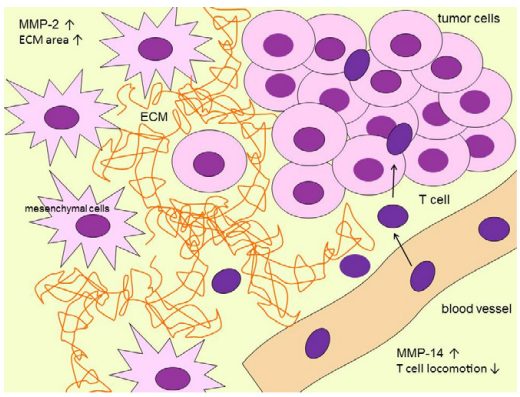

Figure 1: Schematics of tumor formation and interaction with its environment, including T-lymphocyte trafficking from the vessels into stroma and tumor epithelium.

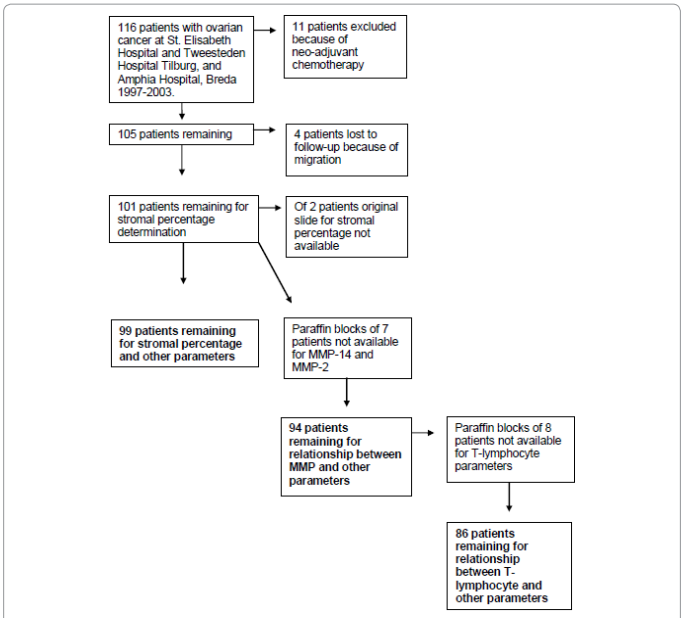

Figure 2: Flowchart of patients. Out of 116 patients, 86 patient biopsies were available for analysis.

when indicated by clinical stage and frozen-section results. Debulking surgery was found to be optimal if the maximum diameter (length, width or depth) of the individual residual tumor deposits were less than 1 cm. In patients with FIGO stage Ia or Ib ovarian cancer with differentiation grade I, no adjuvant therapy was given. All other patients received 6 to 9 courses of adjuvant platinum-based chemotherapy. The only treatment change during the study period was in adjuvant polychemotherapy. In 1999, the combination of cisplatin and cyclophosphamide was changed to carboplatin or cisplatin combined with paclitaxel. FIGO stage (I to IV) was categorized according to World Health Organization (WHO) criteria. Survival data were collected from the medical records, hospital-registered death certificates or registration by the general practitioner of the patient.

Histopathological data

All histopathological results and original slides were reviewed by an independent pathologist, specialized in gynecological pathology. Histology and differentiation grade were categorized according to WHO criteria, grading was based on the observer's impression of both architectural and cytological features [23]. In the original hematoxylin eosin slides, both the areas of tumor epithelium and stroma percentage were quantified from three random images taken adjacent to the epithelial tumor fields (Figure 3) and averaged according to Labiche et al. [21]. Necrotic or adenofibromatotic components of the tumor were excluded from the percentage calculation.

Semiquantitative immunohistochemistry

Paraffin-embedded blocks were selected from the archives of the histopathology laboratory and immunohistochemistry was performed as described before [13]. In brief, sections of 3 µm thickness were deparaffinized in xylene and rehydrated with graded alcohol. Each slide included positive controls for MMP's consisting of placenta. Each run included negative controls without primary antibody. Endogenous peroxidase was blocked with 3% H_2O_2 and 5% normal goat serum. The primary antibodies used for staining are described in Table 1. After each incubation step, slides were washed two times with phosphate buffered saline (PBS).

Slides were incubated with secondary antibody, poly-HRP-GAM/R/R IgG Powervision (Immunologic, Duiven, the Netherlands) for 60 minutes at room temperature. Staining was done with diaminobenzidine (Immunologic, Duiven, the Netherlands) for 5 minutes. Slides were counterstained with hematoxylin.

Scoring of immunostaining

The scoring system according to Kamat et al. used for MMP-14 and MMP-2 incorporated intensity (0 = absent, 1 = weak, 2 = moderate, 3 = strong staining) and percentage of positive tumor cells (0 = 0%, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100% of cells) [16]. Two investigators scored all slides (MCV, EB). If they disagreed, consensus was reached by consulting the gynaecopathologist (AAW).

T-lymphocyte count was recorded according to Zhang et al. [19]. In short, T-lymphocytes were counted in three fields of the tumor compartment and three for the stromal compartment and averaged. CD3 was used as a pan-T-lymphocyte marker. CD8 was used as a cytotoxic T-lymphocyte marker as the presence of CD8 positive cytotoxic lymphocytes is most important for tumor cell killing [19]. The total amount of intratumoral T-cells consist of the CD3 positive cells. CD3 positive cells minus CD8 positive cells will make up the helper T-lymphocyte compartment. Of the helper T-lymphocyte compartment, the regulatory T cells with CD45Ro as a regulatory T-lymphocyte marker, seem to be the most interesting for prognosis [24]. For these markers, the same fields were counted as the fields in the slides used for CD3 determination. In order to make our result comparable to the results of Zhang et al. on clinical characteristics, we followed their methodology in the number and selection of fields and markers despite newer insights into the markers for regulatory T cells [19,25].

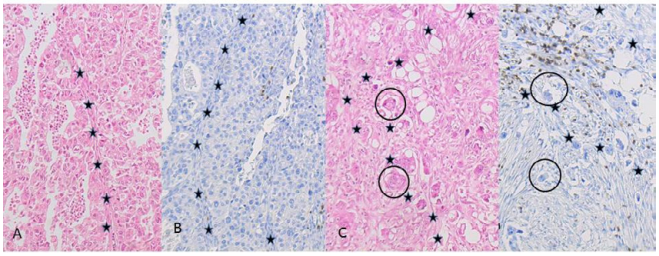

Figure 3: Panel of representative slides of A) ovarian carcinoma with 5% stroma (haematoxylin-eosin staining), B) ovarian carcinoma with 5% stroma (CD3 staining), C) ovarian carcinoma with 85% stroma (haematoxylin-eosin staining), D) ovarian carcinoma with 85% stroma (CD3 staining). The small stromal compartment in A and C and the abundant stromal compartment in B and D is indicated with stars and tumorepithelium in panels C and are encircled. CD3 positivity in B and D is indicated by brown-grey staining.

Antigen	Type	Antibody	Dilution	Incubation
MMP-2*	monoclonal	Clone A-Gel vc2, Thermo Scientific	1:10	4°C overnight
MMP-14**	polyclonal	Thermo Scientific	1:20	Room temperature 60 minutes
CD3	monoclonal	Clone LN10, Monosan	1:80	Room temperature 30 minutes
CD8	monoclonal	Clone C8/144B, Thermo Scientific	1:40	Room temperature 30 minutes
CD45Ro	monoclonal	Clone UCHL1, Biogenex	1:80	Room temperature 30 minutes
*[38], **[39]				

Table 1: Antibodies for immunohistochemistry.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences 20.0 (SPSS Inc., Chicago, Il). Clinicopathological and survival data and immunohistochemistry scores of early-stage and advanced-stage patients were compared using independent samples t-tests for continuous variables if normally distributed, Mann-Whitney tests if continuous variables were not distributed normally and chi-square tests for categorical variables.

To determine the correlation coefficients between MMP-14 and MMP-2, T-lymphocytes and stromal volume, Spearman's rho correlation coefficients were calculated; a value of 0.1 being considered a small correlation, 0.3 medium and 0.5 a high correlation.

Results

Of the 101 patients in this consecutive series from a regional laboratory, 33 were early-stage patients (FIGO Ia-IIa). The median follow-up time was 59 months (minimum 0- maximum 195). Patient characteristics including histopathological subtypes reflect the distribution pattern of diagnosis in the Netherlands 1989-2009 [1]. The patient characteristics are summarized in Table 2. Because the pathophysiology of early-stage ovarian cancer and advanced-stage ovarian cancer are different, we present the results for the two groups in the tables seperately. To relate MMP staining to epithelial tumor or stromal areas and determine the number of T- cell infiltrates, firstly, MMP immunohistochemistry was performed and quantified (Figures 3 and 4) (Table 3 upper part). The MMP-14 and MMP-2 expression in the tumor epithelium was positive (Overall Score 1) in 53/94 and 84/94 of all patients (early and late stage together), respectively, and in the tumor stroma of 56/94 and 30/94 patients (with staining intensity scored weak or strong, respectively).

Second, Table 3 (lower part) shows the median T-cell count for the different markers. For the T-lymphocyte parameters, a large range of counts was observed for both the tumor epithelium and the stromal compartment. T-cells counts were recoded into < 5/High Power Field (HPF), 6-20/HPF and > 20/HPF according to Zhang [19]. Approximately half of the patients had five or less positive T-lymphocytes per HPF in the tumor epithelium (58.1% for CD45Ro, 58.1%for CD8 and 50.0% for CD3). In the stromal compartment, more CD45Ro, CD8 and CD3 positive T-lymphocytes were present. Only 27.9%, 32.6% respectively 19.8% of patients had five or less positive T-lymphocytes in the stromal compartment of the tumor. More T-cells were found –both epithelial and stromal- in advanced stage patients compared to early-stage patients, but only for cytotoxic T-cells (CD8) in stroma this difference was significant.

Third, in early-stage patients, the median percentage of stroma was 40% and in advanced-stage patients 30% (Table 3). Figure 3 illustrates different examples of a tumor with a low and a tumor with a high percentage of stroma. In the lower panels, the slide with CD3 immunostaining is presented.

In Figure 4 different examples of stroma are illustrated with A) necrotic and hemorrhagic stroma, B) more fibrillary and dense stroma and C) normal stroma with inflammatory cells.

Median overall survival for advanced-stage patients was 25

(minimum 0-maximum 191) months in the group of patients with five or less CD3 positive cells versus 47 (minimum 3-maximum 174) months in those with greater than five CD3 positive cells. For CD45Ro, median overall survival was 33 (minimum 0-maximum 191) and 40 (minimum 3-maximum 174) months, respectively, and for CD8 28 (minimum 0-maximum 191) and 46 (minimum 3-maximum 158)

	Early-stage (n = 33)		Advanced stage (n = 68)		p-value
	Median (min-max)	Frequency	Median (min-max)	Frequency	
Age	49(25-73)		60(31-88)		p < 0.001
Histology					p < 0.000
Serous		6		34	
Mucinous		15		3	
Endometroid		4		15	
Clear cell		3		4	
Adenocarcinoma unspecified		3		12	
Mixed		2		0	
Differentiation					p < 0.000
Low-grade		18		10	
High-grade		15		58	
CA 125	24(0-4325)		333(0-44438)		p < 0.000
Ascites					p < 0.000
Absent		25		16	
		7		52	
FIGO stage					
IIB				13	
III				46	
IV				9	
Cytoreduction (only advanced stage disease)					
Optimal				29	
Suboptimal				31	
Progression Free Survival	120(0-195)		18(0-191)		p < 0.000
Overall Survival	121(0-195)		36(0-191)		p < 0.000

Table 2: Patient characteristics.

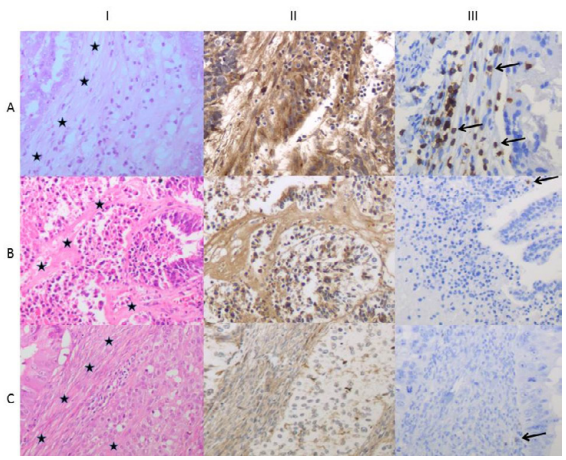

Figure 4: Panel of representative slides of A) normal stroma with inflammatory cells(arrowheads), B) fibrillar and dense stroma and C) dense and more heterogeneous stroma with series of I haematoxylin-eosin staining, II MMP-14 staining (brown) and III CD3 staining (dark brown) (10x10 magnification). Stromal compartments in the slides are indicated with stars and CD3 positive inflammatory cells with arrowheads.

	Number of patients		
	Early stage (n=30)	Advanced stage (n=64)	p-value
MMP-14 tumor epithelium			
Overall Score 0	10	31	ns
Overall Score 1	20	33	ns
MMP-14 stroma			
no	12	26	ns
weak	3	4	ns
strong	15	34	ns
MMP-2 Overall Score			
Overall Score 0	2	9	ns
Overall Score 1	28	55	ns
MMP-2 stroma)			
no	17	47	ns
weak	2	4	ns
strong	11	13	ns
T cell counts	(median (min-max))		
CD45Ro in tumour epithelium	2.5 (0.0-70.3)	3.7 (0.0-181.3)	ns
CD8 in tumour epithelium	3.5 (0.0-47.0)	3.7 (0.0-174.7)	ns
CD3 in tumour epithelium	3.5 (0.0-60.3)	5.7 (0.0-251.2)	ns
CD45Ro in stroma	10.8 (0.0-145.3)	16.5 (0.0-110.7)	ns
CD8 in stroma	6.8 (0.0-88.3)	10.8 (0.0-112.3)	p<0.048
CD3 in stroma	10.5 (0.0-98.0)	20.8 (0.0-208.7)	ns
	(median (min-max))		
Stroma percentage	40 (5-80)	30 (1-95)	ns
Abbreviations: MMP: Matrix Metalloproteinase; CD: Cluster of Differentiation, ns: not significant			

Table 3: Results of immunostaining with p= values between early-stage and advanced stage using Mann-Whitney U tests.

months, respectively. These differences were not statistically significant, but in line with the previous reported results on survival [19].

If the stroma percentage is dichotomized at the median of 30% for advanced-stage patients, median overall survival is 19 (minimum 0-maximum 174) months for patients with <30% stroma and 44 (minimum 6-maximum 191) months for patients with ≥30%. Also, this difference is not statistically different.

Correlation between MMP-14 and MMP-2 expression, T-lymphocytes and stromal volume

Contrary to our original hypothesis that MMP-14 expression in the tumor would positively correlate with T-cell influx, we found that epithelial MMP-14 expression negatively correlated with the presence of T-lymphocytes and yielded correlation coefficients rho -0.36, $p < 0.001$ for CD3; rho -0.30, $p < 0.005$ for CD8; and rho -0.24, $p < 0.03$ CD45Ro (Table 4). No significant correlations between stromal MMP-14 and MMP-2 and the presence of T-lymphocytes in the stromal compartment were found.

Neither significant correlations were found for MMP-2 expression in the tumor epithelium nor in the stroma on T-cell influx. Interestingly, high MMP-2 in stroma significantly correlated with high stroma percentage (rho 0.29, $p < 0.008$), which is also contrary to our original hypothesis.

In conclusion, MMP-14 expression in the tumor epithelium negatively correlates with T-cell influx and stromal MMP-2 expression correlates with the amount of stroma.

Discussion

In this study, we investigated the relationships between MMP-

	CD45Ro tumor expression	CD8 tumor expression	CD3 tumor expression	percentage stroma
MMP-14 tumor epithelium	−0.24*	−0.30**	−0.36**	0.17
MMP-14 stroma	0.04	−0.17	−0.11	−0.05
MMP-2 tumor epithelium	−0.10	−0.15	−0.09	0.04
MMP-2 stroma	0.00	0.02	0.03	0.29**

*Correlation is significant at the 0.05 level (2-tailed)
**Correlation is significant at the 0.01 level (2-tailed)
Abbreviations: MMP: Matrix Metalloproteinase; CD: Cluster of Differentiation

Table 4: Spearman's rho correlation coefficients for MMP-parameters and T-lymphocyte parameters and stroma percentage.

14 and MMP-2 expression in the tumor epithelium and the stromal compartment, together with the presence of T-lymphocytes and the amount of stroma in a regional cohort of ovarian cancer patients. We found a significant negative association between MMP-14 expression within the tumor epithelium and the presence of intratumoral T-lymphocytes. In addition, high stromal MMP-2 expression was positively correlated with stroma percentage. No other significant relationships were found.

It is challenging to interpret our results from a pathophysiological viewpoint. The ovaries are a unique environment to study relationships between MMP parameters and stromal factors. Most ovarian cancers probably do not arise in the ovaries themselves, [26,27] and in terms of disease characteristics, ovarian tumors are strikingly different to tumors in other organs. The main distinguishing features of ovarian tumors are their often cystic nature, their large size at detection and the prolonged confinement of metastases to the peritoneal cavity.

Regarding stroma percentage, our results are not fully comparable with those in the study by Labiche et al. [21]. For advanced stage, the median stroma percentage was 30% in our group and 50% in their group. In early-stage tumors, we found a median percentage of 40%, while early stage tumors were not included in their study. This may be attributable to the different populations that the patients come from, ours being a large regional cohort and theirs from a university referral center, resulting in a possible selection bias. In line with the larger stroma percentage we found in early-stage patients, the relationship between stroma percentage and survival was also different than found in Labiche's study. In our group of advanced stage patients, those with a stroma percentage larger than the median percentage of 30 had a longer survival, although not statistically significant. Perhaps this longer survival goes along with overall less formed tumor mass in relation to the stroma mass. Also, perhaps is more stroma mass a result of less MMP-2 production which regulates part of the, though uncontrolled, stroma turnover. In line with this, in a study on MMP-2 blockade and stroma formation in skin cancer, even though the amount of stroma relative to tumor epithelium was not described in that study, an inhibitory effect on vessel formation and invasion was found [28], reasoning for a better overall survival.

We found a negative association between epithelial MMP-14 expression and T-lymphocyte presence, indicating a role for tumor-expressed MMP-14 in inhibiting T-lymphocyte trafficking towards the tumor epithelium. Our result of the inverse effect of MMP-14 expression on T lymphocyte presence is controversial. For T-lymphocyte infiltration, structural characteristics of malignant stroma are important, in particular, how the arrangement of collagen fibers facilitates or hinders T-lymphocyte trafficking, as shown in an example of lung cancer. When treated with collagenases, T-lymphocyte movement towards the tumor increases. This is in favor of the concept of partial degradation and thus widening of the collagen network for increased T-cell migration [29]. More T-lymphocyte infiltration, especially CD3+CD8+ lymphocytes will result in a better prognosis for the patients [25]. In our advanced-stage patients, we found significantly more CD8+ lymphocytes in stroma. In another example in lung cancer, lymphoid structures containing a reticular network are sometimes located far from the tumor at the boundary between tumor stroma and healthy tissue [3]. In ovarian cancer, this boundary is often less clear, because of the lack of an invasive front by microscopic detection. However, also other adverse roles of MMP-14 are possible. Studies on genetic profiling in ovarian cancer suggest that some tumor types with a mesenchymal, and thus MMP-14-positive profile have less lymphocyte infiltration than other tumor types such as high graded serous and endometroid tumors [7]. From this perspective, high MMP-14 expression in an ovarian tumor may reflect a mesenchymal profile and thus contribute to less T-lymphocyte infiltration, for yet unknown reasons. Unfortunately, analyzing different histopathological groups in our sample will lead to a too small sample size to draw any conclusions.

Comparison of our results with those of earlier studies on T-lymphocytes yields the following conclusions. Zhang et al, established the influence of the presence of tumor-infiltrating lymphocytes on prolonged survival in ovarian cancer [19]. This is in line with our results. However, the underlying mechanisms for this finding are poorly understood.

The roles that MMP-14 and MMP-2 play in malignant tumor development are largely unknown. From the studies on normal ovaries [8,30] and on auto-immune disease [31,32] MMP-14 expression strength should positively correlate with T-cell infiltration, but negatively correlate with stroma percentage. The findings in our presented study show opposite results with the presence of MMP-14 in the tumor epithelium correlating negatively with T-lymphocytes in the tumor epithelium. No relation between MMP-14 and stroma percentage is found, only for stromal MMP-2 a positive correlation is found. The results from the present study thus suggest that in the ovarian environment, the roles of MMP-14 and MMP-2 in malignant stroma are quite different from the roles that MMP-14 and MMP-2 may play in healthy ovarian stroma with an expected positive correlation between MMP-14 and T-cell infiltration and a negative correlation between MMP-2 and stroma percentage [3].

In auto-immune disease, MMP-14 and MMP-2 are important for T-lymphocyte influx via CD44, the integrin pathways and TGF-β [31,33-35]. In ovarian cancer, we found in contrast a negative correlation between the presence of the different types of T-lymphocytes and MMP-14 expression. It seems logical to assume that the degrading action of MMP's on the ECM would facilitate T-lymphocyte infiltration of the tumor stroma followed by infiltration of the tumor mass, but clearly other actions of MMP-14 on signaling pathways are more important. It is possible that chemotactic signals in the micro-environment of the tumor could be more important than the arrangement of the collagen fibers [29,33]. The negative association of MMP-14 on T-lymphocyte influx is in accordance with the negative effect of MMP-14 expression and survival [16]. This negative correlation that we have found would favor the use of MMP-14 inhibitors, which have been, however, already been reported to be ineffective in advanced ovarian cancer [36]. Since correlation coefficients of this MMP-14/T-cell presence relationship are around 0.30, indicating a moderate effect, other factors must also play a role, like maybe CD44 and TGF-β [33,37].

The main strength of the present study is that it is based on a large regional cohort with long-term follow-up. We only excluded patients who had undergone neo-adjuvant chemotherapy, believing that including them might adversely influence the MMP staining results [22].

This study demonstrates the importance of characteristics of malignant stroma in ovarian cancer and the significant role for MMP-14 in T-lymphocyte influx. Clearly, further research is necessary to determine the specific actions of MMP's in ovarian tumor stroma, as well as their interactions with other molecules in this context.

Acknowledgement and Author Contributions

All authors declare no conflict of interest. This research was not funded.

MCV designed the study, scored the immunoassays, performed statistical analysis and drafted the manuscript. EB carried out and scored the immunoassays under supervision of EdB. BdO supervised statistical analysis. AW conceived of the study, supervised scoring the immunoassays and helped to draft the manuscript. TvK, KW and LM supervised the study and helped to draft the manuscript. All authors read and approved the final manuscript.

References

1. van Altena AM, Karim-Kos HE, de Vries E, Kruitwagen RF, Massuger LF, et al. (2012) Trends in therapy and survival of advanced stage epithelial ovarian cancer patients in the netherlands. Gynecol Oncol 125: 649-654.
2. Moserle L, Casanovas O (2013) Anti-angiogenesis and metastasis: A tumour and stromal cell alliance. J Intern Med 273: 128-137.
3. Peranzoni E, Rivas-Caicedo A, Bougherara H, Salmon H, Donnadieu E (2013) Positive and negative influence of the matrix architecture on antitumor immune surveillance. Cellular and Molecular Life Sciences 70: 4431-4448.
4. Kato N, Takeda J, Fukase M, Motoyama T (2010) Alternate mucoid and hyalinized stroma in clear cell carcinoma of the ovary: Manifestation of serial stromal remodeling. Mod Pathol 23: 881-888.
5. Sounni NE, Noel A (2013) Targeting the tumor microenvironment for cancer therapy. Clin Chem 59: 85-93.
6. Wei S, Conner MG, Zhang K, Siegal GP, Novak L (2010) Juxtatumoral stromal reactions in uterine endometrioid adenocarcinoma and their prognostic significance. Int J Gynecol Pathol 29: 562-567.
7. Tothill RW, Tinker AV, George J, Brown R, Fox SB, et al. (2008) Novel molecular subtypes of serous and endometrioid ovarian cancer linked to clinical outcome. Clin Cancer Res 14: 5198-5208.
8. Holmbeck K, Bianco P, Caterina J, Yamada S, Kromer M, et al. (1999) Mt1-mmp-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover. Cell 99: 81-92.
9. Afzal S, Lalani EN, Poulsom R, Stubbs A, Rowlinson G, et al. (1998) Mt1-mmp and mmp-2 mrna expression in human ovarian tumors: Possible implications for the role of desmoplastic fibroblasts. Hum Pathol 29: 155-165.
10. Hotary KB, Yana I, Sabeh F, Li XY, Holmbeck K, et al. (2002) Matrix metalloproteinases (mmps) regulate fibrin-invasive activity via mt1-mmp-dependent and -independent processes. J Exp Med 195: 295-308.
11. Hotary KB, Allen ED, Brooks PC, Datta NS, Long MW, et al. Membrane type i matrix metalloproteinase usurps tumor growth control imposed by the three-dimensional extracellular matrix. Cell 114: 33-45.
12. Langers AM, Verspaget HW, Hawinkels LJ, Kubben FJ, van Duijn W, et al. (2012) Mmp-2 and mmp-9 in normal mucosa are independently associated with outcome of colorectal cancer patients. Br J Cancer 106: 1495-1498.
13. Vos MC, van der Wurff AA, Last JT, de Boed EA, Smeenk JM, et al. (2014) Immunohistochemical expression of mmp-14 and mmp-2, and mmp-2 activity during human ovarian follicular development. Reprod Biol Endocrinol 12: 12.
14. Brun JL, Cortez A, Commo F, Uzan S, Rouzier R, et al. (2008) Serous and mucinous ovarian tumors express different profiles of MMP-2, -7, -9, MT1-MMP, and TIMP-1 and -2. Int J Oncol 33: 1239-1246.
15. Adley BP, Gleason KJ, Yang XJ, Stack MS (2009) Expression of membrane type 1 matrix metalloproteinase (mmp-14) in epithelial ovarian cancer: High level expression in clear cell carcinoma. Gynecol Oncol 112: 319-324.
16. Kamat AA, Fletcher M, Gruman LM, Mueller P, Lopez A, et al. (2006) The clinical relevance of stromal matrix metalloproteinase expression in ovarian cancer. Clin Cancer Res 12: 1707-1714.
17. Brun JL, Cortez A, Lesieur B, Uzan S, Rouzier R, et al. (2012) Expression of MMP-2, -7, -9, MT1-MMP and TIMP-1 and -2 has no prognostic relevance in patients with advanced epithelial ovarian cancer. Oncol Rep 27: 1049-1057.
18. Trudel D, Desmeules P, Turcotte S, Plante M, Grégoire J, et al. (2014) Visual and automated assessment of matrix metalloproteinase-14 tissue expression for the evaluation of ovarian cancer prognosis. Mod Pathol 22: 1394-1404.
19. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, et al. (2003) Intratumoral t cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med 348: 203-213.
20. Horvath LE, Werner T, Boucher K, Jones K (2013) The relationship between tumor size and stage in early versus advanced ovarian cancer. Med Hypotheses 80: 684-687.
21. Labiche A, Heutte N, Herlin P, Chasle J, Gauduchon P, et al. (2010) Stromal compartment as a survival prognostic factor in advanced ovarian carcinoma. Int J Gynecol Cancer 20: 28-33.
22. Karam AK, Santiskulvong C, Fekete M, Zabih S, Eng C, et al. (2010) Cisplatin and pi3kinase inhibition decrease invasion and migration of human ovarian carcinoma cells and regulate matrix-metalloproteinase expression. Cytoskeleton (Hoboken) 67: 535-544.
23. Silverberg SG (2000) Histopathologic grading of ovarian carcinoma: A review and proposal. Int J Gynecol Pathol 19: 7-15.
24. Shah CA, Allison KH, Garcia RL, Gray HJ, Goff BA, et al. (2008) Intratumoral t cells, tumor-associated macrophages, and regulatory t cells: Association with p53 mutations, circulating tumor DNA and survival in women with ovarian cancer. Gynecol Oncol 109: 215-219.
25. Nelson BH (2008) The impact of t-cell immunity on ovarian cancer outcomes. Immunol Rev 222: 101-116.
26. Piek JM, Verheijen RH, van Diest PJ (2009) Tubal and ovarian pathways to pelvic epithelial cancer: A pathological perspective. Histopathology 54: 494-495.
27. Massuger L, Roelofsen T, Ham M, Bulten J (2010) The origin of serous ovarian cancer may be found in the uterus: A novel hypothesis. Med Hypotheses 74: 859-861.
28. Woenne EC, Lederle W, Zwick S, Palmowski M, Krell H, et al. (2010) Mmp inhibition blocks fibroblast-dependent skin cancer invasion, reduces vascularization and alters VEGF-A and PDGF-BB expression. Anticancer Res 30: 703-711.
29. Salmon H, Franciszkiewicz K, Damotte D, Dieu-Nosjean MC, Validire P, et al. (2012) Matrix architecture defines the preferential localization and migration of t cells into the stroma of human lung tumors. J Clin Invest 122: 899-910.
30. Goldman S, Shalev E (2009) Mmps and timps in ovarian physiology and pathophysiology. Front Biosci 9: 2474-2483.
31. Savinov AY, Strongin AY (2007) Defining the roles of t cell membrane proteinase and cd44 in type 1 diabetes. IUBMB Life 59: 6-13.
32. Savinov AY, Strongin AY (2013) Targeting the t-cell membrane type-1 matrix metalloproteinase-cd44 axis in a transferred type 1 diabetes model in nod mice. Exp Ther Med 5: 438-442.
33. Krstic J, Santibanez JF (2014) Transforming growth factor-beta and matrix metalloproteinases: Functional interactions in tumor stroma-infiltrating myeloid cells. TheScientificWorldJournal 2014: 521754.
34. Graesser D, Mahooti S, Madri JA (2000) Distinct roles for matrix metalloproteinase-2 and alpha4 integrin in autoimmune t cell extravasation and residency in brain parenchyma during experimental autoimmune encephalomyelitis. J Neuroimmunol 109: 121-131.
35. Jenkins G (2008) The role of proteases in transforming growth factor-beta activation. Int J Biochem Cell Biol 40: 1068-1078.
36. Nemunaitis J, Poole C, Primrose J, Rosemurgy A, Malfetano J, et al. (1998) Combined analysis of studies of the effects of the matrix metalloproteinase

inhibitor marimastat on serum tumor markers in advanced cancer: Selection of a biologically active and tolerable dose for longer-term studies. Clin Cancer Res 4: 1101-1109.

37. Elliott RL, Blobe GC (2005) Role of transforming growth factor beta in human cancer. J Clin Oncol 23: 2078-2093.

38. Margulies IM, Höyhtyä M, Evans C, Stracke ML, Liotta LA, et al. (1992) Urinary type iv collagenase: Elevated levels are associated with bladder transitional cell carcinoma. Cancer Epidemiol Biomarkers Prev 1: 467-474.

39. Sato H, Takino T, Okada Y, Cao J, Shinagawa A, et al. (1994) A matrix metalloproteinase expressed on the surface of invasive tumour cells. Nature 370: 61-65.