

Chemokine Profile Is Different in Normal Testis Compared to Seminoma – Especially in Tumor Infiltrating Lymphocytes

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Abstract. *Background/Aim:* Testicular cancers, particularly seminomas and non-seminomas, generally have a favorable prognosis, although a small subset of patients experience mortality. Current knowledge of clinical markers associated with relapse and poor prognosis in seminoma is limited. Chemokines, key proteins in the tumor microenvironment, are underexplored in seminoma prognosis. Additionally, tumor-infiltrating lymphocytes (TILs), which play a critical role in cancer prognosis, require further investigation in the context of seminoma. *Patients and Methods:* Samples from 25 seminoma patients and 24 control patients who underwent orchiectomy were immunohistochemically (IHC) stained for chemokines CXCR4, CXCR5, and their ligands CXCL12, CXCL13, and the proliferation marker Ki-67. The associations between IHC results and clinical presentations were examined. *Results:* Chemokine profiles differed between seminoma and normal testis. The expression of chemokines in TILs in seminoma samples was especially over-expressed. The cytoplasmic expression of CXCL13 in TILs multiplied by the percentage of TILs in each

sample, appeared to approach statistical significance concerning the likelihood of relapse. *Conclusion:* The involvement of TILs in seminoma biology warrants further investigation, especially their role in the tumor micro-environment and pathogenesis. Chemokine and Ki-67 expression in TILs could serve as potential markers for assessing seminoma prognosis.

Testicular cancer is the most common malignant tumor in men aged 25-35, accounting for 1% of all cancers (1). The occurrence of testicular cancer has increased remarkably in the past 40 years (2); however, its prognosis is extremely good, with a cure rate close to 100% in non-metastatic disease (3). Testicular cancer often presents as a painless mass in the testicle. Patients may also present with symptoms reminiscent of orchitis or epididymitis, such as swelling or pain and discomfort in the pelvic area (2).

The most widely recognized risk factor for testicular cancer is cryptorchidism (4). Around 90-95% of testicular cancers originate from germ cells, which are referred to as testicular germ cell tumors (TGCTs). They can be further categorized histologically into seminomas and non-seminomas. Seminomas consist of gonocyte-like altered germ cells (4). Seminomas are classified as pure and are generally thought to contain no elements of other histological subtypes (5).

Tumor infiltrating lymphocytes (TILs) are histologically an integral part of seminoma, creating lymphoid aggregates (6). They are vital in restricting and surveilling tumor growth and proliferation. Greater numbers of TILs seem to be associated with a less aggressive clinical presentation in various carcinomas, including seminoma. The presence of TILs is thought to correlate with the efficacy of immune checkpoint blockade (ICB) medication (7). The idea of immune cells playing a vital role in cancer prognosis has been increasingly

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posited over the last few years (8). T-lymphocytes seem to be the most prominent and best identified immune cells within the carcinoma microenvironment (9).

The testis is a distinctive immunological environment due to the blood-testis barrier and its intricate mechanisms maintaining immune defense (10, 11). Serum beta-human chorionic gonadotropin (β -hCG), alpha-fetoprotein (AFP), and lactate dehydrogenase (LDH) are used during diagnostic procedures and during the follow up of testicular cancer to evaluate prognosis and treatment outcomes (2, 3). High levels are also thought to be associated with a higher probability of relapse. Of these markers, increased AFP production occurs only in non-seminomas; therefore, pure seminomas should not present with elevated AFP. Another potential biomarker that could be used in seminoma prognosis is the cytokine MIF (Macrophage migration inhibitory factor), which has been shown to be related to higher stage in TCGT (12). A minority of cases have refractory disease and the possibility of excess mortality, even though seminoma and testicular cancer have an excellent prognosis. More predictive biomarkers are needed to categorize high-risk patients who need adjuvant therapies and those who would benefit more from a surveillance policy. The histopathological assessment of the tumor sample, namely tumor size and the extent of testis invasion, currently seems to be the most reliable way to predict clinical outcome, rather than serum tumor markers (13). The preoperative evaluation of inflammatory markers could be a useful tool to predict the clinical presentation of seminoma (14).

Chemokines are a class of proteins characterized by low molecular weight and the ability to function as chemoattractants, hence being able to influence the migration of adjacent cells (15). They play a pivotal role in, *e.g.*, the regulation of inflammation, and their function is abnormal in many chronic conditions, such as autoimmune disorders. Certain chemokines can assist a cancer's proliferation and growth within its microenvironment (15). The ligands CXCL12 and CXCL13 and their receptors CXCR4 and CXCR5 have been detected in seminoma (16-18), but their clinical significance needs further exploration.

Ki-67 expression is highly associated with cancer cells' proliferation and growth (19). Ki-67 has commonly been used as an indicator of seminoma's proliferative activity (20), but its role in seminoma's clinical implications seems unclear (21). In addition it is commonly used as a prognostic and predictive marker in many cancers, and its expression seems to correlate with both metastasizing and poor cancer cell differentiation (19).

This study's purpose was to assess the chemokine profile in seminoma and the differences between seminoma and normal testes. The study design was based on our previous studies (22, 23). Another point of interest was how the expression of chemokines and Ki-67 in tumor cells and TILs affected the clinical presentation of seminoma.

Table I. Clinical information of the seminoma group, n=25.

	n (%)
Age	
<40 years	19 (76.0)
\geq 40 years	6 (24.0)
Stage	
I	22 (88.0)
II	3 (12.0)
B-symptoms	
None	25 (100.0)
Yes	0
LDH	
Elevated	17 (68.0)
Normal	6 (24.0)
NA	2 (8.0)
AFP	
Elevated	1 (4.0)
Normal	24 (96.0)
hCG	
Elevated	4 (16.0)
Normal	21 (84.0)

LDH: Lactate dehydrogenase; AFP: alpha fetoprotein; hCG: human chorionic gonadotropin; NA: not available.

Patients and Methods

Patients. The study population comprised 25 male patients with de novo seminoma, diagnosed and treated in the Northern Ostrobothnia Hospital district. The control samples included 24 normal/non-neoplastic testes; the purposes of their orchiectomy included palliative surgical castration due to prostate adenocarcinoma (n=16), exploration surgery for undefined tumor (n=3), unidentified cause of testicular pain (n=3), and not known (n=2). All patients and control patients underwent orchiectomy as the initial treatment, and all seminoma patients had a TNM stage I-II disease. Additional treatment options in the seminoma group included radiation therapy (n=11), chemotherapy (n=12), and regional lymphadenectomy (n=1). The chemotherapy comprised carboplatin (n=7) and cisplatin, etoposide, and bleomycin (PEB) (n=5). Two patients were not given any additional treatment regimens. Table I presents the patient demographics. The patients' median age was 36 years (range=22-77 years). The patients' median age in the control group was 74.5 years at the time of orchiectomy (47-85 years). The clinical information collected from hospital records included age, date of diagnosis, follow-up status, date of relapse, given treatment, presence of general symptoms, TNM stage, serum tumor markers (LDH, AFP, β -HCG), s-classification, cryptorchidism, microlithiasis and the IGCCCG-classification.

This study was approved by the Ethical Committee of North Ostrobothnia's Hospital District (251/2019). This study followed the principles of the Declaration of Helsinki.

Immunohistochemistry. The diagnostic biopsy samples were used for immunohistochemical (IHC) analysis. The same IHC procedure and antibodies were used as previously reported (22, 23), and the IHC staining was performed on CXCL12, CXCL13, CXCR4, CXCR5, and Ki67.

Statistical analysis. The IBM SPSS Statistics for Windows tool (IBM, Armonk, NY, USA) was used for statistical analysis. Survival analysis was performed using the Kaplan–Meier analysis. Overall survival (OS) was defined from the date of diagnosis to the last follow-up date or death, whichever came first. Progression-free survival (PFS) was calculated from the date of diagnosis to the last follow-up or the date of the histopathological confirmation of relapse or progression, whichever came first. Interval variables were turned into binary index variables using the ROC curve method. Crosstabulation chi-square test and Fisher’s exact test were used to detect statistically significant associations between index variables.

Histoscore variables were created to turn marker expression levels into more representative values as previously reported (23). Correlation was calculated using bivariate correlations *via* the Pearson test. Correlations above 0.4 and under –0.4 with a *p*-value less than 0.05 were considered significant. Hazard ratios (HR) and confidence intervals (CI) were calculated using the Cox regression model. The criterion for statistical significance was *p*-value <0.05.

Results

Expression of immunohistochemical markers. All tested IHC markers were expressed in seminoma samples (Table II, Figure 1). In normal testicular samples, lymphocytes showed very little CXCL12 expression, with only two samples showing weak expression. In contrast, none of the seminoma samples were positive for CXCL12 in TILs. Weak cytoplasmic CXCL12 expression was observed in 23/23 seminiferous cells in normal samples, whereas in seminoma samples, four presented weak expression and one showed strong expression.

CXCL13 showed weak nuclear expression in TILs in 8/24 samples and strong expression in 3/24 normal samples. In seminoma samples, weak and strong nuclear expression was observed in 20 out of 25 and 19 out of 25 samples, respectively. Normal samples did not express cytoplasmic CXCL13 in lymphocytes and neither nuclear nor cytoplasmic CXCL13 in seminiferous cells. Seminoma cells showed weak cytoplasmic expression in TILs in 18/25 samples. CXCL13 showed weak nuclear expression in 8/25 seminoma cell samples and weak cytoplasmic expression in 5/25 seminoma cell samples.

Regarding CXCR4 immunoreactivity in TILs, one sample showed weak nuclear expression in the normal group, whereas two samples showed weak nuclear expression in the seminoma group. Regarding membranous expression, 22/24 of normal samples and 25/25 of seminoma samples showed weak expression. None of the normal samples presented strong expression, whereas strong expression was found in 7/25 seminoma samples. Normal samples were associated with higher Histoscore (*p*<0.001).

Regarding CXCR5, 8/19 of normal samples showed weak membranous expression in TILs and 5/19 showed strong expression. In seminoma samples, the figures were 19 out of 25 for weak expression and 6 out of 25 for strong expression. Weak membranous expression of CXCR5 in

seminiferous cells was observed in 9 out of 23 non-seminoma samples, while none of the seminoma samples showed membranous CXCR5 expression in seminoma cells.

The quantity of TILs in the non-neoplastic samples ranged from 0–8% (median 1%); while in seminoma samples, TILs ranged from 1 to 25% (median 5%) of the total cellularity. The seminoma samples were associated with a higher number of TILs (*p*=0.005).

The present study found several statistically significant correlations between different cytokines. CXCR5 membranous Histoscore was correlated with CXCR4 nuclear Histoscore in TILs (*r*=0.418, *p*=0.005). The CXCR4 membranous multiplied Histoscore was correlated with the Histoscore value of membranous CXCR5 in TILs (*r*=0.519, *p*<0.001). It also correlated with the Histoscore of nuclear CXCL13 Histoscore in TILs (*r*=0.424, *p*=0.002).

Associations with clinical presentation. Regarding associations with clinical markers, the cytoplasmic expression of CXCL13 in TILs, multiplied by the percentage of TILs in each sample, reached statistical significance in relation to the likelihood of relapse (*p*=0.093). Higher nuclear expression of CXCL13 in TILs was nearly significantly associated with elevated LDH levels (*p*=0.059). Neither of the serum markers, including elevated hCG, LDH, nor age greater than 40 were statistically significantly associated with a higher probability of relapse. These markers were also not significantly associated with any chemokine or Ki-67 expression.

Survival correlations. The mean follow-up time was 109.6 months (range=20–160 months), and the median time from diagnosis to relapse was 10.5 months (range=8–13 months). The number of relapses was two in the present cohort. The 5-year PFS rate in seminoma patients was 91.7 % and the 5-year OS was 100%. Figure 2 presents the PFS rate with regard to cytoplasmic CXCL13 in TILs. The 5-year PFS rate in the high cytoplasmic CXCL13 group was 75%. The HR was not statistically significant (*p*=0.442, 95%CI=0.000–4200).

Discussion

This study revealed new information about TILs in the biology of seminoma and may offer valuable insights for further studies on this topic. A limited number of studies on this subject have been conducted, especially on seminomas, as opposed to testicular germ cell tumors in general. The cytoplasmic expression of CXCL13 in TILs, multiplied by the percentage of TILs, approached statistical significance regarding the likelihood of relapse.

Research on seminomas related to chemokines and Ki-67 is limited. CXCR4 is frequently over-expressed among chemokine receptors in lung, prostate, and breast cancers and it is believed to direct metastasis to locations with high concentrations of

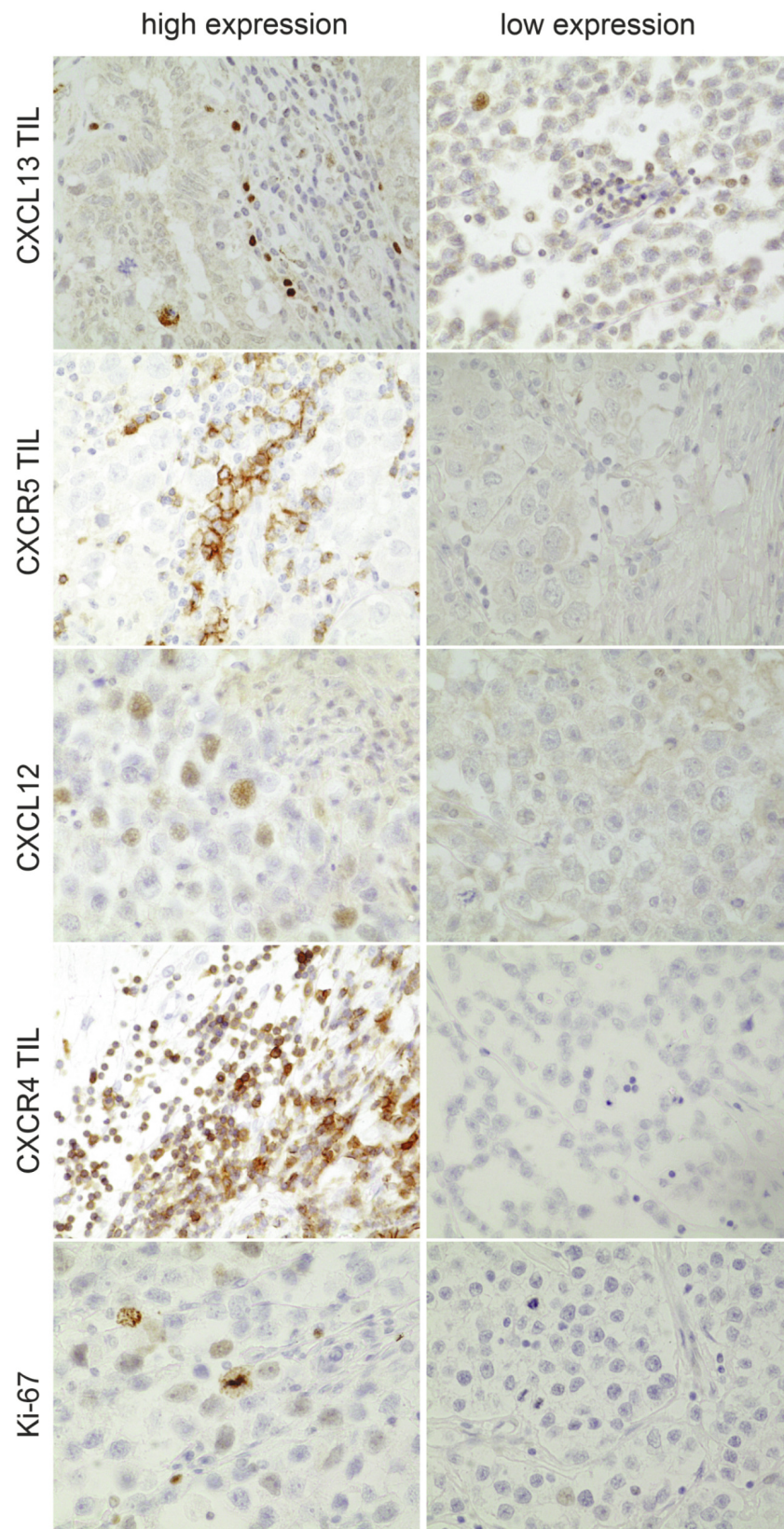


Figure 1. The representation of markers stained in this study at 40× magnification.

Table II. The expression of immunohistochemical markers in different cell structures of seminoma.

Marker	Cytoplasm			Nuclei			Membranes		
	Negative	Weak	Strong	Negative	Weak	Strong	Negative	Weak	Strong
CXCR4	-	-	-	-	-	-	25/25	0/25	0/25
CXCR4*	-	-	-	23/25	2/25	0/25	0/25	25/25	7/25
CXCR5	-	-	-	-	-	-	25/25	0/25	0/25
CXCR5*	-	-	-	-	-	-	6/25	19/25	6/25
CXCL12	20/25	5/25	1/25	25/25	0/25	0/25	25/25	0/25	0/25
CXCL12*	-	-	-	-	-	-	25/25	0/25	0/25
CXCL13	21/25	4/25	0/25	17/25	8/25	0/25	25/25	0/25	0/25
CXCL13*	7/25	18/25	0/25	2/25	20/25	19/25	-	-	-
Ki-67	-	-	-	13/23	10/23	-	-	-	-

*Tumor infiltrating lymphocytes.

CXCL12, such as the bone marrow (18). This characteristic has also been hypothesized to be present in seminoma. The CXCL12-CXCR4 ligand-receptor axis seems to play a crucial role in the homing of certain stem cells and can influence the metastatic destination of tumor cells during embryo development (24). Additionally, CXCL12 and its receptors can act as targets for the transcription factor ETV5 (18). This pathway leads to an increase in the proliferation of spermatogonial stem cells while suppressing their differentiation. Lower expression of CXCL12 has previously been associated with organ-restricted tumors and a reduction in relapse likelihood in TGCTs (16). Seminoma patients with high levels of CXCR4 expression had a worse prognosis in another published study (25). However, CXCL12 expression was not associated with relapse in all testicular carcinoma patients, although CXCL12 seemed to be associated with an improved, relapse-free-survival in non-seminomas (13). CXCL12 is predominantly found in non-seminomas, which may partly explain these results. CXCR4 and CXCL12 also seemed to be commonly co-expressed, as indicated by the current study, which found a correlation between cytoplasmic CXCL12 and membranous CXCR4 in TILs. However, the expression of these markers did not seem to be associated with tumor stage.

According to the prevailing evidence, the CXCL13-CXCR5-axis seems to control the infiltration of lymphocytes in the tumor microenvironment, which in part plays a role in the efficacy of certain cytotoxic cancer medications (15). Common serum markers (AFP, hCG, LDH) incorporated in the surveillance protocol did not seem useful in the early detection of relapse in stage I seminoma patients (26). These serum tumor markers might be more useful in non-seminoma patients. A previous study (17) found a significant increase in CXCL13 expression in testicular germ cell neoplasia, as well as a slight increase in testes without neoplastic cells but with lymphocytic infiltration.

Another interesting pathway in the pathophysiology of cancer is the PD-1-CXCL13-axis. Programmed death 1 (PD-1) is a vital

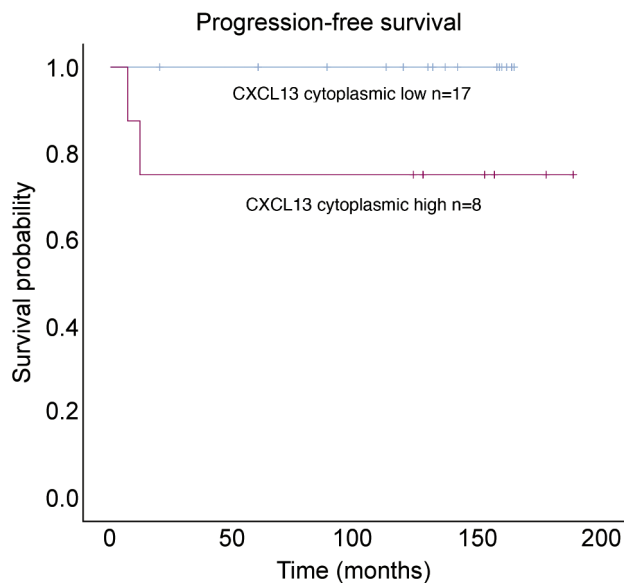


Figure 2. Progression-free survival grouped by cytoplasmic CXCL13 in tumor infiltrating lymphocytes (TILs) multiplied by the percentage of TILs ($p=0.033$).

regulator of peripheral self-tolerance, leading to the apoptosis of unwanted T-cells (27). Anti-PD-1 medication increased OS and PFS in patients with melanoma and lung cancer (27). Additionally, higher expression of PD-1 in TILs seems to lead to a worse clinical manifestation in a handful of tumor types. CD8+ lymphocytes that express high amounts of PD-1 have high levels of CXCL13 secretion, at least in non-small cell lung cancer (15). This pathway seems to increase the number of immune cells directed to the tumor microenvironment, thereby enhancing the efficacy of anti-PD-1-therapies and being associated with an extended OS. Seminomas are characterized by a significant presence of CD8+ T-cells expressing PD-1. Moreover, greater

TIL densities are associated with a lower stage and an absence of lymphovascular invasion at diagnosis (28). Additionally, a higher neutrophil-to-lymphocyte ratio was associated with a higher likelihood of relapse and metastasis in seminoma and NSGCT (29).

Ki-67 and seminoma metastasis at the point of diagnosis do not correlate (21). Furthermore, a recent study (30) found that efficacy of Ki-67 as a useful biomarker for clinical practice in TCGTs. The MKI67 hub gene seemed to be up-regulated in seminoma in another study (31). More research on Ki-67 is needed on this topic.

Study limitations. The study population of seminoma patients was small, representing a pilot study, and these results cannot be extrapolated to a larger population. There were only two cases of relapse within the seminoma group with no mortality, partly due to the comparatively favorable clinical presentation of seminoma. Additionally, the study was retrospective in nature. The extent of available clinical information was limited to the amount gathered during the initial diagnosis, *e.g.*, other serum markers, such as testosterone and thyrotropin, were unavailable at the time of diagnosis. Furthermore, the expression of the studied chemokines was detected in limited quantities in the samples.

Future research on TILs is needed to determine their potential in the biology of seminoma. The properties of the tumor microenvironment significantly impact the prognosis and clinical features in cancer patients, although the extent of this of this influence in seminoma is uncertain. Additionally, more accurate indicators for identifying relapse-prone seminoma are needed.

This study has identified several potential targets for future exploration. The role of Ki-67 in seminoma and its relation to TILs could be further tested, especially in clinical practice. Serum tumor markers could be more expedient in terms of clinical efficacy, because measuring tumor markers in pathological samples could be more costly and time-consuming.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

Conceptualization, M.E.L.K.; methodology, M.E.L.K.; software, J.M.H. and M.E.L.K.; validation, M.E.L.K.; formal analysis, J.M.H., H.T. and M.E.L.K.; investigation, J.M.H., H.T. and M.E.L.K.; resources, J.M.H. and M.E.L.K.; data curation, J.M.H., H.T. and M.E.L.K.; writing – original draft preparation, J.M.H.; writing – review and editing, H.T., K.H., R.K.O., J.K., H.K., O.K., and M.E.L.K.; visualization, M.E.L.K.; supervision, M.E.L.K.; project administration, M.E.L.K.; funding acquisition, M.E.L.K. All Authors have read and agreed to the published version of this manuscript.

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References

- Chung P, Warde P: Testicular cancer: seminoma. *BMJ Clin Evid* 2011: 1807, 2011.
- Motzer RJ, Agarwal N, Beard C, Bhayani S, Bolger GB, Buyyounouski MK, Carducci MA, Chang SS, Choueiri TK, Gupta S, Hancock SL, Hudes GR, Jonasch E, Kuzel TM, Lau C, Levine EG, Lin DW, Margolin KA, Michaelson MD, Olencki T, Pili R, Ratliff TW, Redman BG, Robertson CN, Ryan CJ, Sheinfeld J, Wang J, Wilder RB, National Comprehensive Cancer Network: Testicular Cancer. *J Natl Compr Canc Netw* 10(4): 502-535, 2012. DOI: 10.6004/jnccn.2012.0050
- Oldenburg J, Fosså SD, Nuver J, Heidenreich A, Schmoll HJ, Bokemeyer C, Horwich A, Beyer J, Kataja V, ESMO Guidelines Working Group: Testicular seminoma and non-seminoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 24: vi125-vi132, 2013. DOI: 10.1093/annonc/mdt304
- Hanna NH, Einhorn LH: Testicular Cancer – Discoveries and Updates. *N Engl J Med* 371(21): 2005-2016, 2014. DOI: 10.1056/NEJMra1407550
- Aschim EL, Haugen TB, Tretli S, Daltveit AK, Grotmol T: Risk factors for testicular cancer – differences between pure non-seminoma and mixed seminoma/non-seminoma? *Int J Androl* 29(4): 458-467, 2006. DOI: 10.1111/j.1365-2605.2005.00632.x
- Sakai Y, Hoshino H, Kitazawa R, Kobayashi M: High endothelial venule-like vessels and lymphocyte recruitment in testicular seminoma. *Andrology* 2(2): 282-289, 2014. DOI: 10.1111/j.2047-2927.2014.00192.x
- Liu Y, Liu Z, Yang Y, Cui J, Sun J, Liu Y: The prognostic and biology of tumour-infiltrating lymphocytes in the immunotherapy of cancer. *Br J Cancer* 129(7): 1041-1049, 2023. DOI: 10.1038/s41416-023-02321-y
- Bruni D, Angell HK, Galon J: The immune contexture and Immunoscore in cancer prognosis and therapeutic efficacy. *Nat Rev Cancer* 20(11): 662-680, 2020. DOI: 10.1038/s41568-020-0285-7
- Boldrini R, De Pasquale MD, Melaiu O, Chierici M, Jurman G, Benedetti MC, Salfi NC, Castellano A, Collini P, Furlanello C, Pistoia V, Cifaldi L, Terenziani M, Fruci D: Tumor-infiltrating T cells and PD-L1 expression in childhood malignant extracranial germ-cell tumors. *Oncoimmunology* 8(2): e1542245, 2018. DOI: 10.1080/2162402X.2018.1542245
- Fijak M, Meinhardt A: The testis in immune privilege. *Immunol Rev* 213: 66-1, 2006. DOI: 10.1111/j.1600-065X.2006.00438.x
- Zhao S, Zhu W, Xue S, Han D: Testicular defense systems: immune privilege and innate immunity. *Cell Mol Immunol* 11(5): 428-437, 2014. DOI: 10.1038/cmi.2014.38
- Hauser S, Kaminski A, Syring I, Holdenrieder S, Dieckmann KP, Muller SC, Ellinger J: Evaluation of serum biomarkers (FGF-2,

- HGF, MIF and PTN) in patients with testicular germ cell cancer. *In Vivo* 33(6): 1935-1940, 2019. DOI: 10.21873/invivo.11688
- 13 Lobo J, Gillis AJM, van den Berg A, Looijenga LHJ: Prediction of relapse in stage I testicular germ cell tumor patients on surveillance: investigation of biomarkers. *BMC Cancer* 20(1): 728, 2020. DOI: 10.1186/s12885-020-07220-6
 - 14 Imamoglu G, Eren T, Baylan B, Karacin C: May high levels of systemic immune-inflammation index and hematologic inflammation markers suggest a further stage in testicular tumours? *Urol Int* 103(3): 303-310, 2019. DOI: 10.1159/000502658
 - 15 Kazanietz MG, Durando M, Cooke M: CXCL13 and its receptor CXCR5 in cancer: Inflammation, immune response, and beyond. *Front Endocrinol (Lausanne)* 10: 471, 2019. DOI: 10.3389/fendo.2019.00471
 - 16 Gilbert DC, Chandler I, McIntyre A, Goddard NC, Gabe R, Huddart RA, Shipley J: Clinical and biological significance of CXCL12 and CXCR4 expression in adult testes and germ cell tumours of adults and adolescents. *J Pathol* 217(1): 94-102, 2009. DOI: 10.1002/path.2436
 - 17 Klein B, Haggerty T, Fietz D, Indumathy S, Loveland KL, Hedger M, Kliesch S, Weidner W, Bergmann M, Schuppe HC: Specific immune cell and cytokine characteristics of human testicular germ cell neoplasia. *Hum Reprod* 31(10): 2192-2202, 2016. DOI: 10.1093/humrep/dew211
 - 18 McIver SC, Loveland KL, Roman SD, Nixon B, Kitazawa R, McLaughlin EA: The chemokine CXCL12 and its receptor CXCR4 are implicated in human seminoma metastasis. *Andrology* 1(3): 517-529, 2013. DOI: 10.1111/j.2047-2927.2013.00081.x
 - 19 Li LT, Jiang G, Chen Q, Zheng JN: Ki67 is a promising molecular target in the diagnosis of cancer (Review). *Mol Med Rep* 11(3): 1566-1572, 2015. DOI: 10.3892/mmr.2014.2914
 - 20 Düe W, Dieckmann KP, Loy V: Immunohistological determination of proliferative activity in seminomas. *J Clin Pathol* 41(3): 304-307, 1988. DOI: 10.1136/jcp.41.3.304
 - 21 Gallegos I, Valdevenito JP, Miranda R, Fernandez C: Immunohistochemistry expression of P53, Ki67, CD30, and CD117 and presence of clinical metastasis at diagnosis of testicular seminoma. *Appl Immunohistochem Mol Morphol* 19(2): 147-152, 2011. DOI: 10.1097/PAI.0b013e3181f05a66
 - 22 Chan MC, Savela J, Ollikainen RK, Teppo HR, Miinalainen I, Pirinen R, Kari EJM, Kuitunen H, Turpeenniemi-Hujanen T, Kuitinen O, Kuusisto MEL: Testis-specific thioredoxins TXNDC2, TXNDC3, and TXNDC6 are expressed in both testicular and systemic DLBCL and correlate with clinical disease presentation. *Oxid Med Cell Longev* 2021: 8026941, 2021. DOI: 10.1155/2021/8026941
 - 23 Ollikainen RK, Kotkaranta PH, Kempainen J, Teppo HR, Kuitunen H, Pirinen R, Turpeenniemi-Hujanen T, Kuitinen O, Kuusisto MEL: Different chemokine profile between systemic and testicular diffuse large B-cell lymphoma. *Leuk Lymphoma* 62(9): 2151-2160, 2021. DOI: 10.1080/10428194.2021.1913150
 - 24 Zlotnik A, Yoshie O: The chemokine superfamily revisited. *Immunity* 36(5): 705-716, 2012. DOI: 10.1016/j.immuni.2012.05.008
 - 25 Chen YH, Lin TT, Wu YP, Li XD, Chen SH, Xue XY, Wei Y, Zheng QS, Huang JB, Xu N: Identification of key genes and pathways in seminoma by bioinformatics analysis. *Oncotargets Ther* 12: 3683-3693, 2019. DOI: 10.2147/OTT.S199115
 - 26 Vesprini D, Chung P, Tolan S, Gospodarowicz M, Jewett M, O'Malley M, Sweet J, Moore M, Panzarella T, Sturgeon J, Sugar L, Anson-Cartwright L, Warde P: Utility of serum tumor markers during surveillance for stage I seminoma. *Cancer* 118(21): 5245-5250, 2012. DOI: 10.1002/cncr.27539
 - 27 Menger L, Sledzinska A, Bergerhoff K, Vargas FA, Smith J, Poirot L, Pule M, Herrero J, Peggs KS, Quezada SA: TALEN-mediated inactivation of PD-1 in tumor-reactive lymphocytes promotes intratumoral T-cell persistence and rejection of established tumors. *Cancer Res* 76(8): 2087-2093, 2016. DOI: 10.1158/0008-5472.CAN-15-3352
 - 28 Linder N, Taylor JC, Colling R, Pell R, Alveyn E, Joseph J, Protheroe A, Lundin M, Lundin J, Verrill C: Deep learning for detecting tumour-infiltrating lymphocytes in testicular germ cell tumours. *J Clin Pathol* 72(2): 157-164, 2019. DOI: 10.1136/jclinpath-2018-205328
 - 29 Tan YG, Sia J, Huang HH, Lau WKO: Neutrophil-to-lymphocyte ratio independently predicts advanced pathological staging and poorer survival outcomes in testicular cancer. *Investig Clin Urol* 60(3): 176-183, 2019. DOI: 10.4111/icu.2019.60.3.176
 - 30 Lourenço BC, Guimarães-Teixeira C, Flores BCT, Miranda-Gonçalves V, Guimarães R, Cantante M, Lopes P, Braga I, Maurício J, Jerónimo C, Henrique R, Lobo J: Ki67 and LSD1 expression in testicular germ cell tumors is not associated with patient outcome: investigation using a digital pathology algorithm. *Life (Basel)* 12(2): 264, 2022. DOI: 10.3390/life12020264
 - 31 Wang K, Chen Y, Zhao Z, Feng M, Zhang S: Identification of potential core genes and miRNAs in testicular seminoma *via* bioinformatics analysis. *Mol Med Rep* 20(5): 4013-4022, 2019. DOI: 10.3892/mmr.2019.10684

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