DOI: 10.4081/aiua.2024.12246

ORIGINAL PAPER

Cellular senescence in testicular cancer. Is there a correlation with the preoperative markers and the extent of the tumor? An experimental study

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Summary *Purpose: The aim of this experimental study is to investigate the correlation between the presence of senescent cells and the tumor size, the lymphovascular invasion (LVI), the invasion of rete testis (RTI), the preoperative tumor markers or pathological stage in patients who underwent orchiectomy for malignant purposes.*
Methods: This experimental study included patients with a history of radical orchiectomy performed from January 2011 to January 2019. The testicular tissue specimens underwent an immunohistopathological process for the detection of the presence of cellular senescence. Besides, the tumor size, the histopathological type, the pathological stage of the tumor and the presence of Lymphovascular (LVI) or rete testis (RTI) invasions were also recorded. Additionally, the preoperative serum levels of alpha-fetoprotein, beta-human chorionic gonadotropin and lactate dehydrogenase were recorded. After the completion of immunohistochemical analysis, the rate of senescent cells in each specimen was also recorded.
Results: The mean senescent cell rate was estimated to be 14.11 ± 11.32% and 15.46 ± 10.58% in patients with presence of LVI or absence of LVI, respectively (p = 0.46). The mean senescent cell rate was calculated at 18.13±12.26% and 12.56 ± 9.38% (p = 0.096) in patients with presence of RTI or absence of RTI, respectively. The mean senescent cell rate in the pT1 group was calculated at 14.58 ± 9.82%, while in T2 and T3 groups the mean senescent cell rate was estimated to be 15.22 ± 12.03% and 15.35 ± 14.21%, respectively (p = 0.98). A statistically significant correlation was detected between the senescence rate and the tumor size (Pearson score 0.40, p = 0.027) and between the rate of senescent cells and the preoperative level of lactate dehydrogenase (LDH) (Pearson score -0.53, p = 0.002).
Conclusions: The presence of cellular senescence was correlated with the extent of the testicular tumor in terms of tumor size as well as the preoperative level of the LDH serum marker.

KEY WORDS: *Testicular cancer; Cellular senescence; Preoperative tumor markers; GL13; Senescent cells.*

Submitted 31 December 2023; Accepted 12 January 2024

INTRODUCTION

Testicular cancer is associated with a survival rate of over 95% in 5 years, constituting a solid malignancy with a good prognosis. There are many factors leading to this fact including the young age of the patients, the complete surgical excision of the tumor as well as the effective use of chemotherapeutic agents (1). The staging of testicular cancer plays a crucial role in the successful management of the disease including the use of preoperative tumor markers (2). In clinical practice, *Alpha-fetoprotein* (α-FP), *beta-human chorionic gonadotropin* (β-hCG) and *lactate dehydrogenase (LDH)* constitute the most common serum markers used in staging stratification.
A-FP is a 70kD serum-binding protein that is mainly produced by the yolk sac, liver and intestines. The α-FP levels are peaked between 12-14 weeks of gestation, while they are decreased under 15 ng/ml after the first year of life (3). It has a serum half-life of 5-7 days, while it is considered the most commonly elevated tumor marker in testicular cancer patients (4).
HCG is a 38kD protein that is produced by the placental syncytiotrophoblasts and consists of two subunits (a and b). In contrast to subunit a which resembles the pituitary hormones, β-hCG resembles only 70% of *Luteinizing Hormone* (LH) and thus is easier to be detected.
The β-hCG is characterized by a serum half-life time of 24-36 hours (3, 5).
LDH is a 134kD catalytic agent for the interconversion of lactate and pyruvate. LDH is not associated with high specificity for testicular cancer, nevertheless, it may highlight intense enzymatic processes (3). High levels of LDH over 2.000 U/L seem to be associated with an extremely elevated risk for recurrence (6).
In recent years, the integration of cellular senescence was remarked as a revolution in the field of biology. Interestingly senescence plays a crucial role in tumorigenesis and is considered as hallmark of cancer. It is determined as a special condition of cells and it includes their irreversible growth arrest, metabolism deregulation,

macromolecular damage and a specific secretory phenotype termed *Senescence Associated Secretory Phenotype* (SASP) (7). The cellular senescence could be classified into two types depending on its triggering factors. The first type is widely known as replicative senescence in which the arrest of cellular proliferation is caused by telomere attrition. The second type is stress-induced premature senescence in which stressful stimuli constitute the triggering factors. The role of cellular senescence in the aged skin, in diseases that are determined as age-related and in the procedure of the formation of premalignant lesions is widely studied and presented in the literature. The action of senescence in terms of oncological diseases remains controversial, as it is proven to present both oncosuppressive and oncogenetic characteristics (8). On the one hand, the senescent cells act as tumor-barrier, whereas the accumulation of cells that are vulnerable due to the semi-active metabolic condition may enhance the oncogenesis or the tumor relapses (9).

The association of cellular senescence with visceral tumors or major premalignant structures was investigated, although the available literature is restricted. The role of senescent cells has been described in the frame of a plethora of cancer types such as colorectal, pancreatic and Hodgkin lymphoma (10). Concerning the urogenital region, the presence of cellular senescence in seminomas was detected by *Savelyeva et al.*, while *Majumder et al.* evaluated the presence of senescent cells in *Prostatic Intraepithelial Neoplasia* (PIN) and in prostatic cancer (11, 12). To our knowledge, the impact of cellular senescence in different histopathological types and its correlation with the tumor characteristics was not investigated. The aim of this experimental study is to evaluate the correlation of cellular senescence with the extent of the testicular tumor and with the level of preoperative serum markers.

Materials and methods

Statement of human rights

The ethical committee of the *University of Athens* has approved the conduction of this study in the *"Laiko" General Hospital of Athens*. Informed consent was obtained from all the participants, while the experiments were performed based on the principles of the Helsinki Declaration.

Study design

This experimental study was conducted retrospectively. The study population included patients with a history of radical orchiectomy performed at the *1st Urology Department of Athens Medical School* from January 2011 to January 2019. The radical orchiectomy was performed due to testicular cancer in all the patients. Patients with secondary testicular tumors, incomplete pre- and postoperative data, younger than 18 years old, a history of chemotherapy or patients who were lost during the follow-up were excluded. The investigated samples consisted of *formalin-fixed paraffin-embedded* (FFPE) testicular tissue including tumor, cancerous and premalignant (if available) structures concomitantly and were obtained from the histopathological laboratory. Moreover, the samples were sliced into specimens of 4mm thickness and were stained immunohistochemically against lipofuscin with GL13 (*SenTraGor™*), $p21^{WAF1/CIP1}$ and Ki67, to confirm the presence of senescent cells according to the proposed algorithm (13). GL13 is a biotinylated Sudan Black-B analogue that recognized lipofuscin as a hallmark of senescent cells (8, 14). The $p21^{WAF1/CIP1}$ constitutes a protein that is hardly associated with non-cancerous testicular tissue or *Germ Cell Neoplasia In Situ* (GCNIS) (14, 15). Finally, Ki67 is strongly associated with the growth fraction cells, as it identifies their proliferative activity 16.

Data collection

The preoperative variables included the age of the patient and the date of the operation. The testicular dimensions were recorded based on the report of the pathological examination as it is proven to be the most rigorous measurement (17). Besides the testicular and tumor sizes, the histopathological type and the pathological stage were recorded. The presence of *Lymphovascular Invasion* (LVI) and *Rete testis Invasion* (RTI) presence was also recorded, based on the pathological report.

In the case of mixed tumors, despite the documentation of all the included types and the corresponding rates, the primary histological diagnosis was utilized as the milestone for the patient's subgrouping. Additionally, the preoperative serum levels of α-FP, β-hCG and LDH were recorded. After the completion of immunohistochemical analysis, the total rate of senescent cells in each specimen was also recorded.

Immunohistochemistry for anti-biotin, $p21^{Waf1/Cip1}$ and Ki-67 staining

The samples were deparaffinized and hydrated while the antigen retrieval was heated mediated using 10 mM of citric acid (pH 6.0) for 15 minutes in a steamer (in the case of SenTraGor™ staining). In the case of incubation with $p21^{WAF1/Cip1}$ and Ki67 antibodies, antigen retrieval was completed similarly, but a 25-minute microwave was applied for the heating mediation. Normal goat serum was applied for 1 hour at room temperature (dilution 1:40, Abcam, ab138478) to achieve the blocking of non-specific binding of the aforementioned antibodies. The applied primary antibodies included: anti-biotin antibody (dilution 1:300, Hyb-8, ab201341, Abcam) or anti-$p21^{WAF1/Cip1}$ (dilution 1:200, 12D1, Cell Signaling) or anti-Ki67 (dilution 1:250, Sp-6, Abcam) and the tissues were incubated overnight at 4°C. The use of the *Dako REAL EnVision Detection System kit*, (Cat.no: K5007) according to the manufacturer's instructions using DAB (brown color), led to the progression of the signal. The specimens were counterstained with Hematoxylin to encounter the positive cells.

Finally, the sections were observed using ZEISS Axiolab5 optical microscope on the 20x objective (200x magnification, 25μM scale bar) (13).

SenTraGor™ (GL13) staining for senescence detection

The SenTraGor™ staining was performed following the step of blocking non-specific binding sites of primary antibody. The specimens were incubated in 50% ethanol for 5 minutes and then in 70% ethanol for 5 minutes more. SenTraGor™ was applied in tissues and the speci-

mens were covered with glass coverslips, followed by 10minutes incubation at 37°C. Afterward, coverslips were gently removed and sections were washed with 50% Ethanol for 30 seconds. An extra wash step was performed (3-5 minutes) using Triton-X 0.3%/TBS.
Afterward, the sections were washed with TBS, followed by an overnight application of the anti-biotin antibody (dilution: 1/300, Cat.no: K5007) at 4°C (13).

Statistical analysis

Continuous variables were presented using the mean value and standard deviation. Categorical variables were described as absolute numbers and rates. The comparison of continuous variables was investigated using the two-way t-test, while the correlation between variables was investigated using the Pearson Correlation Coefficient. The differences between multiple groups were evaluated using the one-way ANOVA test. Statistical analysis was conducted utilizing the *Statistical Package for the Social Sciences* (SPSS) software package version 25.0 (*IBM Corp., Armonk, NY*).

Results

In this scientific work in order to evaluate the correlation of senescent cells with the pathological tumor stage, preoperative serum tumor markers, LVI and RTI in different histological types, specimens from 30 patients were utilized. The mean age of the patients was estimated to be 30.97 ± 8.68 years, while the mean maximum diameter of the testis was recorded to be 6 ± 2.80 cm.

Tumor size and cellular senescence

The mean size of tumors in all groups was estimated to be 4.16 ± 3.25 cm. Based on the stratification into groups based on the primary histopathological diagnosis, the larger tumors were identified in the yolk sac groups with a mean maximum diameter of 9.27 ± 4.97 cm, followed by the teratoma group where the mean maximum diameter of the tumor was calculated at 3.83 ± 0.97 cm. The seminomas and embryonal carcinomas were associated with shorter maximum mean diameters (2.9 ± 1.15 cm and 3.46 ± 2.67 cm, respectively). The difference between the groups was statistically significant (p = 0.002). The correlation between cellular senescence and the tumor size overall was statistically significant (Pearson Score: 0.40, p = 0.027). Nevertheless, the correlation in the subgroups did not reach the statistically significant level. More precisely, the Pearson score in the seminoma group was estimated at -0.45 (p = 0.13), while in the embryonal tumor group was calculated at 0.05 (p = 0.89). Additionally, the Pearson scores in the teratomas and yolk sac tumors groups were 0.18 (p = 0.97) and 0.22 (p = 0.86), respectively (Table 1).

LVI and cellular senescence

The LVI was presented in 15/30 (50%) cases, while no LVI was noticed in the remaining 50% of the cases (15/30). More precisely, in 5/13 (38.5%) patients of the seminoma group LVI was detected, while 6/9 (66.7%) patients of the embryonal groups presented with similar pathological characteristics. The LVI was detected in 2/4 (50%) and 2/3 (66.7%) patients in the teratomas and yolk sac tumors, respectively, whereas no LVI was mentioned in the patients with chondrosarcoma. The mean senescent cell rate was estimated to be 14.11 ± 11.32% and 15.46 ± 10.58% in patients with present LVI and absent LVI, respectively.
The difference was not statistically significant (p = 0.46). Moreover, a statistically significant association between the senescent cell rate and LVI was not observed in seminoma (25.98 ± 8.41% *vs* 23.96 ± 6.64%, p = 0.66) or embryonal groups (8.92 ± 7.94% *vs* 4.53 ± 0.93%, p = 0.30) (Table 2).

Table 1.
Evaluation of the senescent cells in relationship to the tumor size.

	Tumor's maximum dameter (mean ± SD)	Senescent cells rate (mean ± SD)	Pearson score	p-value
Overall	4.16 ± 3.25 cm	14.79 ± 10.79%	0.40	0.027*
Seminoma	2.9 ± 1.15 cm	24.74 ± 7.1%	-0.45	0.13
Embryonal	3.46 ± 2.67 cm	7.46 ± 6.82%	0.05	0.89
Teratoma	3.83 ± 0.97 cm	6.9 ± 1.8%	0.18	0.97
Yolk Sac	9.27 ± 4.97 cm	6.06 ± 1.79%	0.22	0.86

SD: Standard Deviation.

Table 2.
Evaluation of the senescent cells in the presence or absence of LVI.

	LVI positive	LVI negative	p-value
Overall			
Patients (%)	15 (50%)	15 (50%)	
Senescent cells rate (mean ± SD)	14.11 ± 11.32%	15.46 ± 10.58%	0.46
Seminoma			
Patients (%)	5 (38.5%)	8 (61.5%)	
Senescent cells rate (mean ± SD)	25.98 ± 8.41%	23.96 ± 6.64%	0.66
Embryonal			
Patients (%)	6 (66.7%)	3 (33.3%)	
Senescent cells rate (mean ± SD)	8.92 ± 7.94%	4.53 ± 0.93%	0.30

LVI: Lymphovascular Invasion; SD: Standard Deviation.

RTI and cellular senescence

The RTI was presented in 12/30 (40%) cases, while no RTI was noticed in the remaining 60% of the cases (18/30). More precisely, in 6/13 (46.2%) patients of the seminoma group RTI was detected, while 4/9 (44.4%) patients of the embryonal groups presented with similar pathological characteristics. The RTI was detected in 1/4 (25%) and 1/3 (33.3%) patients in the teratomas and yolk sac tumors, respectively, whereas no RTI was mentioned in the patients with chondrosarcoma. The mean senescent cell rate was estimated to be 18.13 ± 12.26% and 12.56 ± 9.38% in patients with present RTI or absent RTI, respectively.
The difference was not statistically significant (p = 0.096). Moreover, a statistically significant association between the senescent cell rate and RTI was not observed in seminoma (27.58 ± 5.16% *vs* 22.3 ± 7.97%, p = 0.18) or embryonal groups (9.78 ± 8.16% *vs* 5.6 ± 1.64%, p = 0.54) (Table 3).

Table 3.
Evaluation of the senescent cells in the presence or absence of RTI.

	RTI positive	RTI negative	p-value
Overall			
Patients (%)	12 (40%)	18 (60%)	
Senescent cells rate (mean ± SD)	18.13 ± 12.26%	12.56 ± 9.38%	0.096
Seminoma			
Patients (%)	6 (46.2%)	7 (53.8%)	
Senescent cells rate (mean ± SD)	27.58 ± 5.16%	22.3 ± 7.97%	0.18
Embryonal			
Patients (%)	4 (44.4%)	5 (55.6%)	
Senescent cells rate (mean ± SD)	9.78 ± 8.16%	5.6 ± 1.64%	0.54

RTI: Rete Testis Invasion; SD: Standard Deviation.

Pathological stage and cellular senescence

The association between senescent cell rate and pathological stage of the tumor was also evaluated. In eleven (36.7%), seventeen (56.7%) and two (6.6%) patients the pathological stage was T1, T2 and T3, respectively. The mean senescent cell rate in the T1 group was calculated at 14.58 ± 9.82%, while in the T2 and T3 groups, the mean senescent cell rates were estimated to be 15.22 ± 12.03% and 15.35 ± 14.21%, respectively. The difference did not reach the statistical significance level, as p = 0.98 (Table 4).

Table 4.
Evaluation of the senescent cell rate based on the pathological stage.

	pT1 Stage	pT2 Stage	pT3 Stage	p-value
Sample size (%)	11 (36.7%)	17 (56.7%)	2 (6.6%)	
Senescent cells rate (mean ± SD)	14.58 ± 9.82%	15.22 ± 12.03%	15.35 ± 14.21%	0.98

SD: Standard Deviation.

Preoperative tumor markers and cellular senescence

The correlation between the cellular senescence rate and the preoperative tumor markers constituted another endpoint of the study. In total samples, the correlation between LDH and cellular senescence reached a statistically significant level, as the Pearson score was calculated at -0.53 (p = 0.002). On the other hand, no statistically significant correlation between the rate of senescent cells and the α-FP (Pearson score: -0.24, p = 0.20) or the β-hCG (Pearson score: -0.23, p = 0.23) was detected. After the stratification based on the primary histopathological type, LDH was statistically correlated with cellular senescence only in the seminoma subgroup. The Pearson scores for LDH were estimated to be 0.56 (p = 0.045), -0.04 (p = 0.92), 0.091 (p = 0.90) and -0.06 (p = 0.96) in seminoma, embryonal, teratoma and yolk sac groups, respectively. The outcomes concerning the Pearson scores of α-FP were -0.13, p = 0.66 in the seminoma group; 0.30, p = 0.43 in the embryonal group; 0.39, p = 0.61 in the teratoma group; 0.06, p = 0.96 in the yolk sac group. The correlation between senescent cell rate and the β-hCG did not reach a statistically significant level in any subgroup (Pearson score 0.11, p = 0.72 in the seminoma group; Pearson score 0.56, p = 0.11 in the embryonal group; Pearson score 0.37, p = 0.63 in the teratoma group; Pearson score -0.2, p = 0.87 in the yolk sac group) (Table 5).

Table 5.
The correlation scores between cellular senescence and the preoperative serum tumor markers.

	Pearson Score	p-value
Overall		
LDH	-0.53	0.002*
aFP	-0.24	0.20
b-HCG	-0.23	0.23
Seminoma		
LDH	0.56	0.045*
a-FP	-0.13	0.66
b-hCG	0.11	0.72
Embryonal		
LDH	-0.04	0.92
a-FP	0.30	0.43
b-hCG	0.56	0.11
Teratoma		
LDH	0.091	0.90
a-FP	0.39	0.61
b-hCG	0.37	0.63
Yolk Sac		
LDH	-0.06	0.96
a-FP	0.06	0.96
b-hCG	0.2	0.87

LDH: Lactate Dehydrogenase; a-FP: Alpha-fetoprotein; b-hCG: beta-human chorionic gonadotropin.

Discussion

The aim of this scientific study was to evaluate the association of the senescent cell detection rate with the tumor size, the preoperative tumor markers, the pathological stage and the presence of LVI and RTI. In order to investigate the aforementioned correlation, stains with specific biomarkers showing the state of the examined tissues (proliferation or cellular senescence) were applied. The lipofuscin, which constitutes an established biomarker of senescence, was detected by the GL13 staining (8). The staining pattern in these cells seems to be both perinuclear and cytoplasmic. Besides GL13, $p21^{21WAF1/CIP1}$ and Ki67 stains were utilized to perform a more robust disclosure of the cellular senescence in situ and to elucidate additional elements of the molecular behaviour of the evaluated tissues (18). The outcomes of the study proved that there is a statistical correlation between cellular senescence and the tumor size in overall the sample size, although this correlation was not confirmed in the subgroup analysis. Despite being significantly associated with tumor size, cellular senescence does not seem to be strongly correlated with the pathological stage of the tumors. Additionally, the presence of LVI or RTI does not seem to have a specific association with the senescent cell detection rates. Concerning the preoperative tumor markers, no significant association between cellular senescence and the α-FP or b-hCG was detected in either the overall sample size or subgroups. On the other hand, LDH was statistically sig-

nificantly correlated with the senescent cell detection rates in the overall sample size and seminoma group, whereas no significant correlation with the embryonal, teratoma and yolk sac groups. To our knowledge, this is the first study that investigated the correlation of cellular senescence with the extent of the tumor and the preoperative serum tumor markers.

The impact of cellular senescence in the carcinogenesis of the genitourinary system was documented in both testicular and prostate cancer. The presence of senescent cells in testicular cancer was described by *Savelyeva et al.* (11). The authors investigated the microenvironment of pure seminomas and they identified that it compromises senescent cells (11). Concerning prostate cancer, the role of cellular senescence was investigated by *Majumder et al.* and *Mourkioti et al. Majumder et al.* evaluated the presence of senescent cells in both premalignant conditions of PIN and prostatic cancer tissues. Based on their outcomes, senescent cells were detected in cases of PIN or PIN and prostatic cancer structures concomitantly, whereas no cellular senescence was identified in prostate cancer-only tissues. In our study, cellular senescence was investigated in terms of correlation to the extent of the disease and to the preoperative tumor serum markers in various histopathological types of testicular cancer (12). Moreover, *Mourkioti et al.* proved that the downregulation of CDC6 gene expression may enhance androgen receptor blockade-induced senescence in metastatic prostate cancer cells highlighting the therapeutic impact of cellular senescence even in advanced cancer stages (19). Based on our outcomes, a significant correlation of cellular senescence with the tumor size (Pearson Score: 0.40, $p = 0.027$) and LDH (Pearson Score: -0.53, $p = 0.045$) was identified. Thus, we can confirm the presence of senescent cells in various histological types of testicular cancer, even if the correlation was not statistically significant, as was described by *Savelyeva et al.* (11). Additionally, contrary to *Majumder et al.*, cellular senescence was detected in all histological types of testicular tissue. A possible explanation for that phenomenon could be the different methodology of senescence detection, as GL13 seems to be the most accurate marker for senescence detection since it detects lipofuscin accumulation as a result of the deregulated metabolism, macromolecular damage and cell cycle arrest, the three out of four hallmarks of senescence (14). Moreover, the application of additional senescence markers contributed to an improved evaluation of the tissue structures.

The controversial impact of cellular senescence in oncogenesis was widely evaluated in the literature. Cellular senescence seems to be a barrier to the triggered oncogenes showing its important tumor-suppressive role (18). The cycle arrest condition that characterizes cellular senescence seems to improve the efficacy of the apoptosis or DNA-damage repair processes. On the contrary, senescent cells may accumulate in different types of tissue due to the disturbance of the induction/elimination balance. The aforementioned disturbance leads to the accumulation of senescent cells that are vulnerable to modifications of their genome or SASP. These modifications result in either *endothelial-to-mesenchymal transition* (EMT) or self-renewal which are characterized by invasive/metastatic or stemness potential, respectively (20). Besides the cycle arrest, they seem to escape and re-enter the cell cycle, a phenomenon termed "*Escape from Senescence*". The latter are associated with a different phenotype compared to the primary senescent cells and thus they may be characterized as more aggressive and potentially oncogenetic.

SASP has a paracrine function that promotes cellular senescence and its tumor-suppressive role in low-grade preneoplastic cells increasing the interleukin, chemokine, growth factors and regulator, receptor and ligand levels, and altering the function of collagens, laminin and reactive oxygen species (bright side) (21). On the contrary, it encourages the escape from senescence and relapse to the cell cycle in the malignant cells (dark side) (7). The modifications of the SASP profile are considered the cause of the bimodal role of cellular senescence. Normally, SASP includes interleukins, chemokines, growth factors and additional soluble factors in equilibrium that enhance its tumor-suppressive role. On the other hand, under the impact of p53 loss or oncogenic RAS gain, the SASP is modified compromising abnormally elevated interleukins, chemokines and other inflammatory factors that induce the escape phenomenon. More precisely, in prostate cancer, the secretion of SDF-1α factor in the frame of SASP of senescent fibroblasts seems to enhance the prostate cancer progress, while the hypersecretion of inflammatory factors of senescent cells' SASP may be associated with higher risk for relapse after chemotherapy (21). The latter proves the negative aspect of cellular senescence that is related to the oncological diseases (9, 22). This phenomenon may explain the negative correlation between cellular senescence and the LDH, which has been proved in our study. Senescent cells are normally accumulated in premalignant lesions, as described in prostatic and pancreatic cancer, whereas LDH is elevated in some cases of testicular neoplasia as accompanied by intense enzymatic processes (3, 10, 12). In our study, the negative correlation (Pearson score: -0.53) may indicate the decrease of senescent cell detection rate during the transformation of the premalignant to malignant conditions (which are characterized by elevated LDH) highlighting the escape phenomenon as described by *Zampetidis et al.* (9).

Besides the significant negative correlation between LDH and cellular senescence in overall the sample size, another positive correlation was identified in the seminoma subgroup (Pearson score: 0.56, $p = 0.045$). Thus, we can consider that the senescent cell detection rate remains at relatively high levels in the seminoma group, while it is decreased in the other histopathological subgroups. This observation may be explained by the sequence of testicular cancer as proposed by von *Eyben et al.* (23). The authors supported that the seminoma constitutes the first step of the transformation of the premalignant conditions (GCNIS) to testicular cancer. Afterward, the seminoma may be transformed into embryonal carcinoma, followed by the parallel development of teratoma or yolk sac tumor. Consequently, we can consider that a significant rate of senescent cells remains in the seminoma, which may escape from senescence in the latter steps (9).

There is detailed documentation in the literature regarding the association between senescent cells and colorectal cancer, lung adenocarcinoma, or classical Hodgkin lymphoma (10, 24, 25). Moreover, the role of cellular senes-

cence is also important in the failure of chemotherapy and the disease relapses. The presence of senescent Hodgkin and *Reed-Sternberg* cells in high proportions (over 10-15%) seems to be a risk factor for poor prognosis in patients suffering from Hodgkin lymphoma, as described by *Broeckelman et al.* (26). In our study, the positive correlation of cellular senescence with LDH in the seminoma groups may indicate the presence of a relatively high proportion of senescent cells in the seminomas. Taking into consideration the example of Hodgkin lymphoma, we may consider the management of both the tumor and the senescent cells in specific cases. The combination of chemotherapeutic agents with senolytics may improve the efficacy of the treatment, prevent tumor relapses and ameliorate the prognosis and the quality of life of the patients.

Consequently, novel combined chemotherapeutic strategies aiming at both cancerous and senescent cells may be developed and evaluated.

There are limitations in our study. The sample size is relatively small, as it includes tissue specimens of 30 patients. Besides the reduced sample size, this is the first study to our knowledge that evaluated the correlation between cellular senescence and the extent of testicular cancer or the preoperative tumor markers. Moreover, the outcomes of the immunohistochemical staining are not associated with the clinical presentation and the prognosis of the patients. Further studies should be conducted for the correlation of immunohistochemical and clinical outcomes.

Conclusions

The presence of cellular senescence was correlated with the extent of the testicular tumor in terms of tumor maximum diameter, whereas the association was insignificant regarding the pathological stage and the lymphovascular or rete testis invasions.

The levels of LDH preoperatively seem to be negatively correlated to the presence of cellular senescence, while an additional positive correlation was detected in patients suffering from seminoma.

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Conflict of interest: The authors declare no potential conflict of interest.