Ph.D. Thesis

The prevalence of adult benign teratomas and its comparative molecular (FISH) and immunohistochemical analysis with pediatric and adult malignant cases

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1. Introduction

The 2004 WHO classification divided testicular teratomas into two main groups, prepubertal or pediatric and postpubertal or adult. Prepubertal type is considered benign, whereas postpubertal type with metastizing potential is considered definitely malignant, the minority of the latter is pure and the majority is part of mixed germ cell tumours, Nevertheless, in the recent years two publications – including our – reported benign teratomas in adults (in postpubertal testis) beyond puberty. In spite of this, all teratomas developing in the postpubertal testis (whether histologically mature or immature) are considered malignant in the clinicopathological practice, thus, chemotherapy is indicated depending on the stage according to the current treatment protocol complemented with retroperitoneal lymphadenectomy (RLA) as required, which is exhausting and redundant in the case of a tumour proven to be benign.

1.1 Current WHO classification (2016)

Based on the most recent studies – including those carried out in the Department of Pathology of Pécs University – the current WHO classification has partially corrected the deficiencies of the 2004 version. Moreover, tumours are classified as prepubertal and postpubertal types based on their pathogenetic origin.

1.1.1 Prepubertal teratoma

Whereas postpubertal teratomas derive definitely from malignant germ cells and **GCNIS is virtually always present** in the adjacent, tumour-free testicular tissue; beside the **lack of GCNIS prepubertal type** can be characterized by the appearance of organoid, mature, non-dermal (intestinal, respiratory, tubal, etc.) tissues **without overrepresentation of 12p** that occurs in most testicular germ cell tumours. Moreover, in contrast with malignant cases in adults, tumour markers are consistently negative and no metastases were reported.

Although its name does not indicate it, prepubertal (pediatric) teratoma does not exclusively occur in children and the number of adult cases exceeds pediatric cases in absolute terms according to our research.

1.2. Literature review of IMP3

IMP3 is the third member of the IGF-2 mRNA-binding protein family containing two RNA recognition and four hnRNP K homology domain. The members of the family are encoded by IGF2BP3 gene located at the 7p15.3 region .

IMP3 is almost exclusively expressed in embryonic tissue physiologically and plays an important role in cell migration and early embryogenesis and can be detected in oocytes and granulosa cells in the ovaries and in spermatogonia, spermatocytes and spermatozoa in the testes.

According to some studies IMP3 is not expressed in benign tissues, it acts as an oncoprotein triggering growing, invasion and metastasis formation in advanced stage and agressive tumours. Increased IMP3 expression was detected in renal , gastric , hepatic ,lung, germ cell, colon, pancreatic and ovarian carcinomas. Close correlation was proven between the stage of the disease, the clinical outcome and the IMP3 expression, increased IMP3 expression was associated with worse clinical outcome.

Based on literature data IMP3 seems to play role in the development of GCTs and can be detected with immunohistochemistry in almost all testicular GCTs. The strongest staining was identified in embryonal carcinomas, however considerable differnce can be observed between teratomas in males and females, positive reaction can be detected in primary and metastatic testicular teratomas, no staining can be identified in females and in benign cases, though. The aforementioned results seemed to confirm that the histiogenesis of the vast majority of adult testicular teratomas and probably testicular teratomas differ from that of ovarian teratomas.

2. Materials and methods

2.1 Materials

Examinations were performed on 593 testicular tumours submitted for histologic analysis between 1998 and 2014 at the Department of Pathology (Pécs University, Medical School / Clinical Centre), from which 543 proved to be germ cell tumours.

The material of our department was complemented with selected material of patients from different cities of the country with testicular tumour treated in the National Institute of Oncology between 2000 and 2016 to perform detailed histologic and molecular pathologic

analysis. Pure teratomas were selected based on histologic results out of approximately 5600 testicular tumour cases collected jointly with the National Institute of Oncology and corresponding histology sections and paraffin blocks were requested for further processing from the referring hospital or clinic. Therefore, 36 pure teratoma cases were available for examination.

Approval for research was requested and received from the Regional Ethics Committee. (PTE 47407/2017).

2.2. Fluorescent in situ hybridization

Six mixed, nonseminomatous testicular germ cell tumour cases were used as positive controls and four non-neoplastic tissue samples were used as negative controls in order to avoid fals positive results.

2.4. Light microscopy and immunohistochemistry

New slides were prepared from the samples in order to determine histologic characteristics. Mature and immature tumour components were evaluated (epidermoid cyst, dermoid cyst, intestinal epithelium, other type of epithelium, mesenchyma, neural elements, cartilage, bone, etc.). Tumours that could not be enrolled into the study for any reason were excluded (imprecise diagnosis, not pure teratoma, too small or necrotic tumour, inconclusive sample, etc.). Tumour-free testicular tissue was searched for GCNIS. If GCNIS was not explicit or could not be verified with the analysis of the traditional hematoxylin and eosin staining immunohistochemical examination of PLAP confirmed the presence or lack it.

Additionally, IMP3 immunohistochemical examination was preformed for all cases according to relevant literature.

3. Results

To improve clarity of the text, the attribute prepubertal and postpubertal will not be applied further in the thesis. All malignant cases were postpubertal, so these will be named simply "malignant", whereas benign tumours will be divided into "pediatric" and "adult benign" subsets as no malignant case occurred among pediatric patients and the mean ages of the two populations (1.3 and 28 years) allow this simplification. **Cases with 12p abnormality or the presence of GCNIS were considered malignant teratomas, whereas cases lacking these features were considered benign (Table 1.)**.

After the complete processing and re-evaluation of cases belonging to the Department of Pathology and selected cases received from various Hungarian Institutions 36 pure teratoma cases (14 from the database of Pécs and 22 from the national database) were available. Out of these all adult cases – except benign cases that had previously been evaluated at Pécs University – were diagnosed as malignant. Among pure teratomas 23 proved to be malignant, all of them were adult cases and alltogether 13 proved to be benign composing of 6 pediatric and 7 adult cases.

3.1. Estimating the prevalence of adult benign cases

Prevalence was estimated based on 593 testicular tumours submitted in our department between 1998 and 2014, from which 14 cases (3%) were identified as pure teratomas including one pediatric case. In the material of our department 23% of pure teratoma cases – every fifth, alltogether 3 cases –proved to be benign (not including the only pediatric case). Thus, in the tested sample adult bening teratoma occurred more frequently than the pediatric type.

Case	Age	Size (mm)	12p	GCNIS	IMP3	RLA	Chemo	LN met	Survival (year)
#7	18	13	-	-[1]	-	+	-	-	7
#8	20	22	-	-[1]	-	-	-	-	8
#9	27	8	-	-[1]	-	-	-	-	3
#10	30	15	-	-[1]	-	-	-	-	4
#11	33	20	-	-[1]	-	-	-	-	6
#12	36	10	-	-[1]	-	-	-	-	2
#13	38	10	-	-[1]	NA	NA	NA	NA	7
#14	25	25	+	NA[4]	+	+	+	+	17
#15	21	25	+	+	+	-	-	-	6
#16	32	22	+	+[2]	+	+	-	+	13
#17	41	9	+	+	+	+	+	+	3
#18	21	30	+	+	+	-	-	-	13
#19	22	20	+	NA[4]	+	+	+	-	16
#20	38	60	+	+	+	+	+	+	3
#21	22	30	+	+	+	+	-	-	14
#22	29	19	+	NA[3]	+	+	-	+	9
#23	29	22	+	+	+	-	-	-	4
#24	18	85	+	+	+	+	-	-	4
#25	18	38	+	+[2]	+	-	-	-	4
#26	24	14	+	-	+	-	-	-	3
#27	22	35	+	+	+	-	-	-	4
#28	38	60	+	+	+	+	+	+	3
#29	31	40	+	NA[4]	+	+	+	+	17
#30	43	32	+	NA[3]	+	+	+	+	4
#31	26	18	+	+	+	+	+	+	†
#32	36	36	+	NA[4]	+	+	+	+	3
#33	25	35	+	+	+	+	+	+	4
#34	34	110	NA	+	+	+	+	+	6
#35	33	70	NA	+	+	+	+	+	9
#36	19	54	NA	+	+	-	+	-	14

Table 1: Comparison of adult teratoma cases and the follow up data #7-13 cases: benign teratomas, #14-36: malignant teratomas. 12p: 12p abnormality (FISH), GCNIS: germ cell neoplasia in situ. ND: no data, [1]: PLAP immunohistochemistry was also performed, [2]: only a few PLAP+ intratubular cells were detected, [3]: Sertoli cell only – GCNIS can not be assessed, [4]: no or minimal testicular parenchyma, GCNIS can not be assessed. Chemo: chemotherapy was performed, LN met: lymph node metastasis, †: died

3.2. Histologic composition

Regarding the prevalence of different tissues significant difference was explored between pediatric, adult benign and malignant cases. In the case of pediatric and adult benign tumours difference was only detected in the prevalence of cartilage tissue; it occured in 67% of pediatric

cases, whereas could not been detected in any of the adult cases (Fisher's exact test: p=0.021). Intestinal epithelium and neural elements occured more frequently in pediatric cases (83% vs. 29% and 50% vs. 0%, respectively), however, the difference was not significant (Fisher's exact test: p=0.078 and 0.070, respectively). No considerable difference was detected between these two groups in the prevalence of other tissue elements (Table 2).

Significant difference between pediatric and adult malignant cases was only detected in the prevalence of bone tissue, which was 67% in pediatric cases, whereas only 9% in malignant cases (Fisher's exact test, p=0.008).

Significant difference was detected between adult benign and malignant cases in the prevalence of other epithelium (29% vs. 61%, respectively) and cartilage tissue (0% vs. 52%, respectively) (Fisher'exact test: p=0.006 and 0.016, respectively). No significant difference was found in the prevalence of other tissue elements (Table 2).

Having analysed the histologic diversity of teratomas – i.e. the number of tissue components – it can be concluded that adult benign teratomas contain the lowest number of tissue elements (on average 2.5, SD: 0.5), whereas on average 5.3 (SD: 2.1) different tissue elements occurred in pediatric and 4.0 (SD: 1.4) in malignant cases. The difference between adult benign and malignant cases was significant (t-test, p<0.001).

	Number of cases	Age	Tumour size	Epidermoid cyst	Dermoid cyst	Intestinal epithelium	Other epithelium	Mesenchymal elements	Neural elements	Cartilage tissue	Bone tissue
All cases	36	24	31	53%	8%	58%	83%	100%	25%	44%	22%
Malignant	23	28	39	57%	4%	61%	96%[1]	100%	26%	52%[2]	9%[4]
Benign	13	16	19	46%	15%	54%	62%	100%	23%	31%	46%
pediatric	6	1,3	24	50%	33%	83%	83%	100%	50%	67%[3]	67%[4]
adult	7	29	14	43%	0%	29%	43%[1]	100%	0%	0%[2,3]	29%

Table 2. Clinical and histologic characteristics of the 36 cases examined. Significant difference was detected between pairs marked with superscript. 1: p = 0.006, 2: p = 0.016, 3: p = 0.021, 4: p = 0.008.

3.3.Immunohistochemical analysis of IMP3 protein expression

No specimen was available for further testing in one adult benign case, thus, reaction could not been performed and IMP3 expression was analysed in 35 cases. Intact and GCNIS containing parenchyma were utilized as internal controls.

Out of the 35 analyzed cases all the malignant were definitely positive. Out of the six pediatric benign cases strong staining was detected in five, whereas among the seven adult benign samples no staining was identified in six (using positive internal control) (Table 1). The difference between pediatric and adult benign cases was significant, as well as between adult benign and malignant cases (Fisher's exact test $p<0.001$ for both comparison).

3.4 FISH analysis

3.4.1 Evaluation of control samples

On average, more 12p (G:green) than CEP12 (A:aqua, blue) signals (G>A%) were detected in 15% of cells (± 7%) in normal tissues containing squamous and glandular epithelium used as negative control. The cause of this phenomenon is the truncation of nuclei leading to an unequal loss of FISH-signal occurring randomly, therefore, it can lead to an inverse difference of comparable rate as well in the number of signal spots. The threshold of false positive error was defined as 31% (mean + SD x 2). The mean 12p/CEP12 rate was 1.0 (0.9 – 1.1). Polysomy 12 was present on average in 3% (±2%) of cells, threshold of false positivity was 11%.

The signal pattern of all not pure adult teratoma cases exceeded by far the previously mentioned threshold suggesting polysomy 12 or overrepresentation of 12p.The mean G>A% was 78% (±19), the lowest value was 44% in the case of one sample. Based on our results this parameter distinguishes reliably between cases positive or negative for 12p abnormality. The mean 12p/CEP12 rate was 1.8 (1.3 – 2.5) indicating this parameter is also appropriate to distinguish between positive and negative cases with the application of 1.3 as a threshold. The rate of polysomy was 68% (±50%) (Figure 14B – D and 15).

3.4.2 Analysis of pediatric cases

Neither polysomy nor 12p abnormality was detected in any of the six pure teratoma cases. The mean value was 3% (±1%) in the case of the former and 2% (±1%) in the case of G>A%. The 12p/CEP12 rate ranged between 1.0 – 1.11 in the different cases.

4.5.1. Analysis of adult cases

No evaluable FISH signal could be detected in three out of the thirty adult cases. 12p abnormality similar to that of the positive controls was confirmed in 23 cases. Polysomy 12 was also present apart from one case, polysomy 21 was identified in 19 cases. Neither polysomy 12 nor polysomy 21 occurred without 12p abnormality.

If overrepresentation of 12p or polysomy was present it could be identified in all parts of the tumour including all tissue components comprising that. The mean G>A% was 79% (40-100%), whereas polysomy occurred in 49% (12-80%) on average in positive cases. The rate of cells showing polysomy exceeded the threshold of false positive error in one particular case, however, the number of average CEP12 signals did not exceed two. The latter could be caused by significant truncation effect possibly leading to an underestimation of signals. The average 12p/CEP12 rate was 1.9 (1.3 – 3.1). The rate was 1.3 in three cases that could also be caused by the unequal loss of FISH signal based on the truncation of neuclei.

The average G>A% value was 6% (0 – 24%), the occurrence of the pattern respresentative of polysomy 12 was 4% (0 – 10%) in the seven adult teratomas. Values did not reach the characteristic values of positive controls in any of the cases. The 12p/CEP12 rate ranged between 1.0 – 1.2. (Table 3)

Azonosító	Életkor	12p	21q	CEP12	G>A %	G/A arány	Poliszómia 12	Poliszómia 21
Poz		6,0	3,8	4,4	76%	1,4	84%	80%
Poz	24	4,1	2,7	2,6	84%	1,7	56%	50%
Poz	25	5,0	1,8	2,2	96%	2,5	24%	16%
Poz	33	6,9	3,3	4,1	78%	1,9	86%	66%
Poz	34	6,0	2,4	3,0	92%	2,1	68%	42%
Poz	27	4,4	2,7	4,0	44%	1,3	88%	52%
Neg		1,7	1,6	1,6	14%	1,1	0%	0%
Neg		1,7	1,7	1,8	20%	1,0	8%	4%
Neg		1,6	1,9	1,8	4%	0,9	4%	4%
Neg		1,8	1,8	1,8	20%	1,1	0%	0%
1	0,9	2,1	2,0	2,1	0%	1,0	8%	0%
2	0,4	1,9	1,7	1,8	10%	1,1	8%	0%
3	0,5	2,0	2,0	2,0	0%	1,0	0%	0%
4	0,7	2,0	2,0	2,0	0%	1,0	0%	0%
5	0,4	2,0	2,0	2,0	0%	1,0	0%	0%
6	5	2,0	2,0	2,0	0	1	0%	0%
7	18	1,8	1,6	1,8	24%	1,2	0%	0%
8	20	2,2	1,9	2,2	0%	1,0	10%	0%
9	27	2,0	2,0	2,0	0%	1,0	0%	0%
10	30	2,0	1,9	2,0	10%	1,1	10%	10%
11	33	2,1	2,2	2,1	0%	1,0	6%	6%
12	36	2,0	2,0	2,0	0%	1,0	0%	0%
13	38	1,2	1,0	1,2	8%	1,0	0%	0%
14	25	3,46	2,78	2,74	40%	1,3	48%	58%
15	21	3,2	2,6	2,1	82%	1,8	40%	42%
16	32	4,6	2,6	2,6	92%	2,0	60%	52%
17	41	3,5	1,7	2,8	46%	1,3	50%	0%
18	21	5,0	2,9	3,5	84%	1,5	78%	68%
19	22	4,6	2,7	3,6	50%	1,3	64%	48%
20	38	3,1	1,9	2,0	54%	1,6	12%	12%
21	22	4,0	2,6	2,7	64%	1,7	60%	58%
22	29	4,1	2,2	2,3	72%	2,0	36%	22%
23	29	5,3	2,1	3,2	84%	1,8	68%	40%
24	18	3,8	2,7	2,8	52%	1,4	52%	56%
25	18	5,5	2,0	3,0	96%	1,9	64%	8%
26	24	4,6	1,7	1,8	100%	2,7	8%	12%
27	22	5,4	3,4	2,6	100%	2,3	40%	64%
28	38	7,8	2,8	2,8	100%	2,9	60%	52%
29	31	5,8	2,8	2,9	100%	2,3	52%	56%
30	43	5,4	2,7	3,7	100%	1,6	80%	60%
31	26	3,4	2,2	2,6	68%	1,4	52%	36%
32	36	5,8	2,5	2,3	96%	2,9	36%	40%
33	25	6,0	2,0	2,1	100%	3,1	20%	12%
34	34	NI						
35	33	NI						
36	19	NI						

Table 3. The results of FISH examination. 12p: average ETV6 signals, 21q: average RUNX1 signals, CEP12: average CEP12 signals in a particular sample, based on the evaluation of 50 cells, green: ≤ 2, orange: >2 and < 3, red: ≥ 3; G>A%: percentage of 12p>CEP12 cases, G/A rate: 12p/CEP12 rate, polysomy 12: rate of cells containing more than two CEP12 signals, polysomy 21: rate of cells showing more than two 21q signals, red: exceeds the upper limit of false positive error, green: does not exceed the upper limit of false positive error.

4. Discussion

According to our research testicular teratomas can be divided into three distinct groups. Beside malignant teratomas that are traditionally named postpubertal teratomas, prepubertal benign cases can be divided into pediatric and adult subsets. Beside GCNIS, malignant teratomas are large in size, show 12p abnormality, IMP3 positivity, and occur typically in adulthood. They

frequently contain intestinal and other non-cutaneous epithelium, cartilage tissue and rarely contain bone tissue and those characteristic of dermoid cyst.

GCNIS does not occur in the adult benign group, and these tumours are characterized by IMP3 negativity, the lack of 12p abnormality, small size and rarely contain cartilage or bone tissue, non-cutaneous and non-intestinal epithelium and neural tissue.

Pediatric teratomas are GCNIS negative, middle-sized, IMP3 positive, however do not show 12p abnormality and often contain cartilage, bone tissue and typically contain neural tissue (Table 4).

Teratoma type	Age (yr)*	GCNIS	IMP3	FISH	Size (mm) *	Histologic features
pediatric[1]	0-3	no	yes	nem	17-31	often cartilage, bone tissue, neural tissue is typical
benign[2]	22-36	no	no	nem	9-19	rarely cartilage, bone, other epithelial or neural tissue
malignant[3]	25-31	yes	yes	igen	28-49	rarely bone tissue, often other epithelial or neural tissue

Table 4: Characteristics of different testicular teratomas. *95% confidence interval of age and tumour size are shown. [1]WHO prepubertal type teratoma, [2]Who prepubertal type teratoma in postpubertal testis, [3]WHO postpubertal type teratoma

4.1. The importance of mean age

According to our evaluation pediatric (prepubertal) teratomas typically occur in the first year of life, confidence interval ranges between 0-3 years. One particular sample from a patient older than 3 years was analyzed within this category. Although IMP3 positivity and the presence of cartilage tissue were typical in this group, none of these were identified in the aforementioned case (see below).

4.2. The role of histologic features

Basically differentiated histologic elements were identified in all teratomas, therefore, it can be claimed that the the degree of differentiation does not have prognostic value, although its importance has previosly been emphasized. This statement does not apply for teratomas with secondary somatic malignancy, naturally. The average size of malignant teratomas exceeds that of benign teratomas and pediatric teratomas are larger on average than adult benign ones.

Diagnostic threshold can not be determined, however, no adult benign teratoma larger than 22 mm were identified and only approximately one-fourth of malignant cases did not exceed 22 mm.

GCNIS was only detected in malignant cases, in 90% of them. Nevertheless, approximately in 22% of cases the available histologic sample did not make the identification possible; testicular parenchyma was not visible in three cases, whereas only Sertoli cells were present in the tubules in two cases. Only a minimal amount of GCNIS components were present in approximately 10% of cases leading to an insecure identification if the analyzed parenchyma is too small. The phenomenon is diagnostic, if present, however its lack does not exclude the possibility of a malignant teratoma.

Significant differences were found between the three goups regarding tissue components too. Distinguishing of pediatric teratoma and malignant cases can be supported by the frequent occurrence of bone tissue in the first group. Malignant and adult benign cases differ in the prevalence of intestinal and other non-cutaneous epithelium and cartilage tissue, whereas significant difference was identified in the occurence of dermoid cysts, neural elements and cartilage tissue between pediatric and adult benign teratomas (Table 5). Adult benign teratomas have much less complex histologically than the other two types, as the average number of histologic components was 2.4 in adult benign teratomas, 5.3 in pediatric cases and 4.0 in malignant cases.

4.3. The importance of IMP3 expression

The importance of IMP3 expression is not obvious regarding the slightly controversial data in literature. Positivity of pediatric cases contradict the suggestion that it is not present in benign tumours, however, the negativity of adult benign cases correlates well with the lack of i(12)p and the simultaneous occurrence of these contradict with malignant behaviour. Similar observation was made by Cornejo and colleagues detecting the reaction in 45% of pediatric teratomas. Interestingly, i(12)p was detected in two cases, therefore, it is doubtful, whether these are true benign cases, follow-up data was not published, though.

The positivity of pediatric cases can be explained with the retained ability of pluripotent, primordial germ cells in infants and young children to express IMP3. The only pediatric teratoma case representing IMP3 negativity originated from a 5-year-old child, therefore it, should be considered that – as observed frequently experimentally – the teratoma had lost the ability to express IMP3 during the maturation process, in other words the tumour cells had

„completed" the encoded genetic program and had differentiated to the – otherwise normal – IMP3 state. Therefore, the presence of IMP3 is related to dignity – it is expressed in numerous malignant tumours – and immature histological status as well. Consequently, similarity between pediatric cases and embryonal tissues can explain IMP3 expression, even if positivity can not be found in benign tumours according to the literature.

Based on FISH results, morphology and IMP3 state we conclude that pediatric and adult benign cases are fundamentally the same entity and the observed differences are a result of the encoded genetic program as a part of the „maturation" process completing with age. This hypothesis meets with that of Oosterhuis and Loojitenga, according to which pediatric type I teratoma can rarely occur in adulthood. Nevertheless the idea, that the pediatric and adult benign tumours are separate subtypes can not be ruled out completely. This novel suggestion could be confirmed or refused with independent studies analyzing greater number of cases.

These also mean that the current WHO nomanclature should definitely be reviewed and tumours should be named as „benign" and „malignant" regardless of age.

According to our experiences IMP3 immunohistochemistry definitely promotes distinguishing between adult benign and malignant teratomas, as IMP3 expression correlated well with 12p status determined with FISH. Taking into consideration that immunohistochemistry is more widely applied than FISH, IMP3 analysis could promote correct diagnosis making even in smaller hospitals.

5. Summary of new results

1. The prevalence of adult beningn teratomas has been determined for the first time based on surgical pathologic samples of 17 years. According to our FISH ans IMP3 results every fifth adult pure teratoma case was benign.

2. We pointed out the histologic difference between adult malignant and benign cases. Smaller tumour size, the rare presence of cartilage, bone, other type of epithelium or neural elements and the absence of dermoid cysts and GCNIS can suggest adult benign teratoma.

3. For the first time, adult benign and malignant cases can be distinguished safely with an immunohistochemical marker. The parallel use of IMP3 antibody and FISH examination led to a practically perfect concordance in the identification of adult benign cases. Its use can promote

the safe screening of benign cases even in smaller diagnostic centres, consequently contributing to avoid unnecessary chemotherapy and surgery.

4. Based on our results, we recommended the review of the WHO nomenclature. We believe – in accordance with the relevant literature data – that pediatric and adult benign cases are likely to be the same tumour considering certain histological parameters and IMP3 expression. Despite the facts above, the possibility that the pediatric and adult variants are distinct entities can not be ruled out, therefore further cases have to be evaluated in order to come to a definite conclusion in this issue.

Regardless of which theory is to be confirmed in the future, tumours should merely be described as „benign" and „malignant" based on morphologic, histologic and IMP3 staining status regardless of the patient's age providing straithforward guidance on the treatment to both urologist and oncologist colleauges.

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8. Abbreviations

GCNIS	germ cell neoplasia in situ
GCT	germ cell tumour
IMP3	mitochondrial ribosomal protein S4
PLAP	placental alkaline phosphatase
RLA	retroperitoneal lymphadenectomy
HE	hematoxilin-eozin