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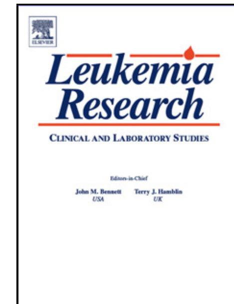

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Sensitive quantification of the intronless SOX11 mRNA from lymph nodes biopsies in mantle cell lymphoma

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Keywords :
SOX11, CCND1, qPCR, lymph nodes, Mantle celle lymphoma (MCL)

To the Editor,
We would like to complement the study of SRY-related HMG-box 11 (SOX11) in blood and bone marrow (BM), which we previously have published in your journal [1], with the investigation of transcript levels in lymph nodes (LN) in mantle cell lymphoma (MCL).

SOX11 is an important diagnostic marker in MCL and assists the classification of two major disease subgroups. The classical nodal MCL displays SOX11 overexpression and unmutated or minimally mutated immunoglobulin V-genes (IGHV). In contrast, the non-nodal MCL is characterized by no or low expression of SOX11, has mutated IGHV and an overall superior prognosis [2, 3]. Sensitive mRNA quantification of SOX11 expression is challenged by the

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intronless nature of the gene with a high risk of contamination with genomic DNA. Contamination from genomic DNA can be partly reduced or eliminated using DNAse. However, performing control reactions for each RNA sample, confirming that no genomic DNA is present, is costly and cumbersome and might not solve the problem [4]. This contributing background noise is detrimental to sensitive and reliable quantification of minimal residual disease. SOX11 has previously been explored in non-malignant B cells from lymphoid tissue by qPCR [5, 6]. However, to our knowledge, these studies did not involve an mRNA-specific methodology taking the technically difficult intronless nature of SOX11 into account and only explored few samples.

In the current study, we investigate the mRNA expression levels of SOX11 and the classical MCL marker Cyclin D1 (CCND1) in i) diagnostic LN from 34 MCL patients (25:9 males to females ratio, median age 67), ii) in peripheral blood mononuclear cells (PBMCs) from 7 healthy donors and iii) in non-malignant tonsils from 21 individuals (10:11 males to females ratio, median age 18) undergoing tonsillectomy. The latter was included as non-malignant B cells from lymphoid tissue, which has also been implemented in previous studies as control material in MCL [7-9]. mRNA was purified from PBMCs isolated using a Ficoll gradient centrifugation and from homogenized LN and tonsil tissues. SOX11 and CCND1 expression was quantified and calculated as reference gene normalized expression values (RGN), using the reference genes GUSB and B2M and defined as relative expression levels per 1000 reference gene transcripts as previously described $((2^{(CqRGmean-CqSOX11 \text{ or } CCND1)})*1000)$[1, 10, 11]. Employing this assay, based on cDNA synthesis using oligo(dT) primer and a poly-T specific reverse PCR primer together with a locked nucleic acid (LNA)-modified hydrolysis probe compensating for the low GC content, there was no risk of genomic DNA amplification [10], thereby excluding a critical methodological issue for the reliable quantification of low levels of transcripts from intronless genes.

In our present study, 33 out of 34 (97 %) MCL LNs expressed a significant high level of SOX11 (1.41-90.84 SOX11 RGN, median: 18.8), while one MCL LN was negative (Fig 1). This SOX11 negative LN was confirmed negative by immunohistochemistry. The healthy donors were consistently negative for SOX11 mRNA expression in PBMCs (Fig 1). Only six out of 21 (28.6 %) non-malignant tonsils expressed SOX11 at a very low level (0.006-0.06, median: 0.05).

CCND1 was expressed in all samples analyzed with the highest expression in MCL LNs (12.8-3396.8 CCND1 RGN, median: 250.1) (Fig 1) and the lowest in healthy donor PBMCs (range 0.10-0.28, median: 0.17,). Of note, the median expression in non-malignant tonsils was 29.1 (range 6.8-99.6), thus considerably higher than the expression in healthy donor PBMCs in this study, and also

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higher than the expression observed in *non-malignant* mononuclear cells from blood and bone marrow in our previous study (PBMCs, median: 0.19 and BM, median: 0.24) [1].

Summarizing, and in stark contrast to SOX11, we found a considerable overlap of the CCND1 expression range between non-malignant tonsils and MCL LNs. This is most likely due to the CCND1 expression in the tonsil microenvironmental cells as it is known that non-neoplastic cells can be positive for CCND1, such as the suprabasal squamous epithelial cells, scattered lymphocytes and endothelial cells showing a moderate to strong distinct nuclear staining reaction by immunohistochemistry [12]. This suggests that SOX11 is a superior marker compared to CCND1 if qPCR is used for minimal residual disease assessment since SOX11 is not or barely expressed in tonsils or PBMCs. The use of CCND1 in these cases would require a B cell enrichment to minimize contamination from CCND1 expressed by microenvironmental cells. However, it is most likely that the characterization of lymph nodes with respect to SOX11 mRNA expression will mainly be of diagnostic value, still, the no or very limited background expression also here remains an advantage.

In conclusion, we demonstrate a high mRNA expression of SOX11 in MCL LNs compared to lymphoid tissue from healthy donors, such as non-malignant tonsils using a highly specific and sensitive qPCR, which corroborates that SOX11 is preferentially used in MCL LNs.

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References:

1. Simonsen AT, Sorensen CD, Ebbesen LH, Bodker JS, Bentzen HH, Nyvold CG: **SOX11 as a minimal residual disease marker for Mantle cell lymphoma**. *Leukemia research* 2014, **38**(8):918-924.
2. Meggendorfer M, Kern W, Haferlach C, Haferlach T, Schnittger S: **SOX11 overexpression is a specific marker for mantle cell lymphoma and correlates with t(11;14) translocation, CCND1 expression and an adverse prognosis**. *Leukemia* 2013, **27**(12):2388-2391.
3. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, Advani R, Ghielmini M, Salles GA, Zelenetz AD *et al*: **The 2016 revision of the World Health Organization classification of lymphoid neoplasms**. *Blood* 2016, **127**(20):2375-2390.
4. Padhi BK, Singh M, Huang N, Pelletier G: **A PCR-based approach to assess genomic DNA contamination in RNA: Application to rat RNA samples**. *Anal Biochem* 2016, **494**:49-51.
5. Ek S, Dictor M, Jerkeman M, Jirstrom K, Borrebaeck CA: **Nuclear expression of the non B-cell lineage Sox11 transcription factor identifies mantle cell lymphoma**. *Blood* 2008, **111**(2):800-805.
6. Lord M, Wasik AM, Christensson B, Sander B: **The utility of mRNA analysis in defining SOX11 expression levels in mantle cell lymphoma and reactive lymph nodes**. *Haematologica* 2015, **100**(9):e369-372.
7. Wang M, Atayar C, Rosati S, Bosga-Bouwer A, Kluin P, Visser L: **JNK is constitutively active in mantle cell lymphoma: cell cycle deregulation and polyploidy by JNK inhibitor SP600125**. *J Pathol* 2009, **218**(1):95-103.
8. Ghobrial IM, McCormick DJ, Kaufmann SH, Leontovich AA, Loegering DA, Dai NT, Krajnik KL, Stenson MJ, Melhem MF, Novak AJ *et al*: **Proteomic analysis of mantle-cell lymphoma by protein microarray**. *Blood* 2005, **105**(9):3722-3730.
9. Martinez N, Camacho FI, Algara P, Rodriguez A, Dopazo A, Ruiz-Ballesteros E, Martin P, Martinez-Climent JA, Garcia-Conde J, Menarguez J *et al*: **The molecular signature of mantle cell lymphoma reveals multiple signals favoring cell survival**. *Cancer research* 2003, **63**(23):8226-8232.
10. Hamborg KH, Bentzen HH, Grubach L, Hokland P, Nyvold CG: **A highly sensitive and specific qPCR assay for quantification of the biomarker SOX11 in mantle cell lymphoma**. *European journal of haematology* 2012, **89**(5):385-394.
11. Simonsen AT, Schou M, Sorensen CD, Bentzen HH, Nyvold CG: **SOX11, CCND1, BCL1/IgH and IgH-VDJ: a battle of minimal residual disease markers in mantle cell lymphoma?** *Leukemia & lymphoma* 2015, **56**(9):2724-2727.
12. Abdulla Z, Turley H, Gatter K, Pezzella F: **Immunohistological recognition of cyclin D1 expression by non-lymphoid cells among lymphoid neoplastic cells**. *APMIS* 2014, **122**(3):183-191.

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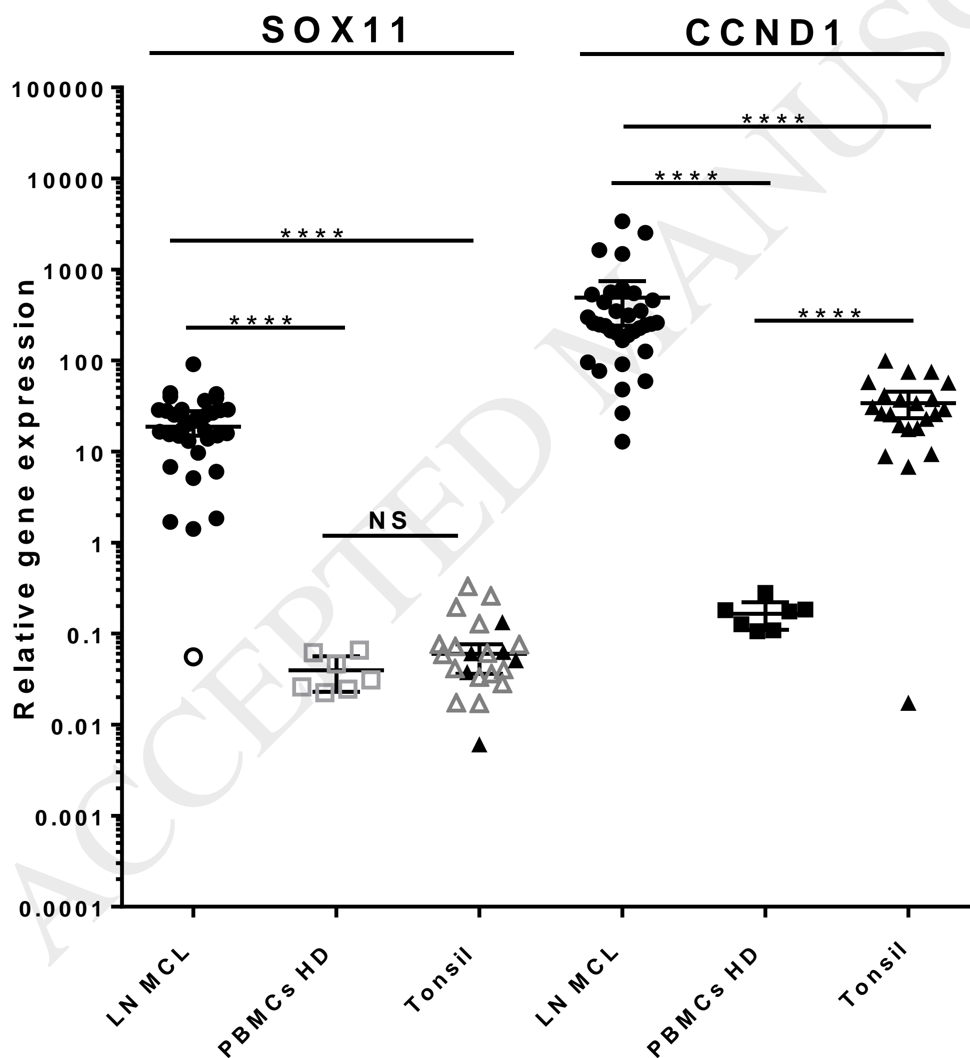
Figure legend for Figure 1:

Relative SOX11 and CCND1 mRNA expression in malignant and control lymphoid tissues

The graph shows the relative SOX11 and CCND1 transcript expression as normalized expression levels per 1000 reference gene transcripts in lymph nodes from MCL patients (LN MCL, circles), PBMCs from healthy donors (PBMCs HD, squares) and non-malignant tonsils (Tonsil, triangles). Open figures denote negative samples. Medians and 95% confidence interval are indicated for each group. For both SOX11 and CCND1 the statistical difference between the control groups and MCL patient group was $p<0.0001$ determined by the Mann-Whitney U test, indicated by ****. The relative mRNA expression was calculated using the 2^{dCq} method with reference genes GUSB and B2M, as previously described [1, 10].

Figure 1



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