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The value of thymus and activation related chemokine immunohistochemistry in classic Hodgkin lymphoma diagnostics

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Aims: Classic Hodgkin lymphoma (cHL) should be distinguished from its wide variety of histological mimics, including reactive conditions and mature B and T cell neoplasms. Thymus and activation-related chemokine (TARC) is produced in extremely high quantities by the Hodgkin/Reed–Sternberg (HRS) tumour cells and is largely responsible for the attraction of $CD4^+$ T cells into the cHL tumour micro-environment. In the current study we evaluated the diagnostic potential of TARC immunohistochemistry in daily practice in a tertiary referral centre in the Netherlands.

Methods and results: A total of 383 cases, approximately half of which were cHL mimics, were prospectively evaluated in the period from June 2014 to November 2020. In 190 cHL cases, 92% were TARC-positive and the majority of cases showed strong and highly specific staining in all HRS cells (77%). In most cases, TARC could discriminate between nodular lymphocyte-predominant and lymphocyte-rich Hodgkin lymphoma. HRS-like cells in mature lymphoid neoplasms were rarely positive (6.4%) and there was no TARC staining at all in 64 reactive lymphadenopathies.

Conclusions: TARC immunohistochemistry has great value in differentiating between cHL and its mimics, including nodular lymphocyte-predominant Hodgkin lymphoma, reactive lymphadenopathies and mature lymphoid neoplasms with HRS-like cells.

Keywords: CCL17, differential diagnostics, Hodgkin lymphoma, immunohistochemistry, TARC

Introduction

Classic Hodgkin lymphoma (cHL) is characterised by the presence of Hodgkin and Reed–Sternberg (HRS) cells in a background of reactive immune cells. Although originating from germinal-centre B cells, HRS cells have lost their B cell phenotype by down-regulation of B cell transcription factors. This is reflected by weak PAX-5 expression and absence of (or weak) CD20 and CD79a expression by immunohistochemistry (IHC). Instead, HRS cells virtually always express CD30 and IRF4/MUM1 and are usually positive for CD15. The cHL tumour micro-environment consists of variable numbers of T cells, B cells, plasma cells, macrophages, eosinophils and neutrophils. T cells are most consistently present and often form so-called rosettes.[1,2] The diagnosis of cHL is straightforward in most cases, but diagnostic difficulties can occur because cHL features may overlap with those of reactive lymphadenopathies, nodular lymphocyte-predominant Hodgkin lymphoma

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(NLPHL) and mature lymphoid neoplasms.[3] These cHL mimics require a different treatment and have a better or sometimes much worse prognosis; for example, in case of angioimmunoblastic T cell lymphoma. Although the majority of cHL cases can be diagnosed reliably by combining morphology, immunohistochemistry and clinical characteristics, there is still room for improvement.

CC chemokine 17 (CCL17), also known as thymus and activation-regulated chemokine (TARC), is a chemokine which is extremely highly expressed and secreted by HRS cells.[4] This expression is much higher than the physiological expression by normal dendritic and epithelial cells of the thymus.[5] TARC binds to the CC chemokine receptor 4 (CCR4) on $CD4^+$ T cells and strongly contributes to the characteristic T cell-rich tumour micro-environment of cHL.[6] Because of the high secretion of TARC by HRS cells, approximately 85% of cHL patients have elevated serum or plasma levels at diagnosis compared to healthy controls (median ~400× higher). Elevated TARC levels correspond with higher-stage disease and tumour volume.[7,8] Previous research from our group and others has also shown that TARC levels in peripheral blood accurately correlate with response to clinical treatment.[9–11]

In earlier retrospective work, approximately 86% of cHL patients showed positive staining of HRS cells for TARC by IHC indicating its potential diagnostic value.[5] Despite these promising results, no study has yet confirmed the value of TARC IHC in daily diagnostic practice.[12] The aim of the current study was to evaluate the diagnostic value of TARC IHC in differentiating between cHL, NLPHL, reactive lymphadenopathies and mature lymphoid neoplasms with HRS-like cells.

Materials and methods

STUDY COHORT AND DATA COLLECTION

This study was performed at the department of Pathology and Medical Biology of the University Medical Center Groningen, a tertiary referral centre covering lymphoma diagnostics in approximately 5 million inhabitants of the Netherlands. TARC IHC was introduced in March 2014 and used by consulting haematopathologists S.R. and A.D. for in-house diagnostics, revisions and consultations. In this prospective diagnostic setting, the governing World Health Organisation (WHO) classification of lymphomas was followed and TARC IHC was used as an addition to the regular diagnostic work-up, including molecular diagnostic T cell clonality analysis to distinguish between cHL and T cell lymphoma when needed.[1] Only the first diagnostic specimen was included in the case of multiple diagnostic procedures in the same patient. The study was conducted in accordance with the Declaration of Helsinki and the medical ethical review board of the University Medical Center Groningen approved the protocol under #RR202100080.

TARC IMMUNOHISTOCHEMISTRY

Paraffin tissue sections (3 μm) were incubated with polyclonal goat anti-human TARC antibody (1:800; R&D Systems, Minneapolis, MN, USA) on the automated Benchmark ULTRA platform (Ultra CC1, 52 min; Roche, Ventana Medical Systems, Oro Valley, AZ, USA). For each TARC stain, a section of cHL tissue was applied on the same slide as an external positive control. Positive TARC staining was defined as cytoplasmic positivity in the HRS cells. We subdivided TARC-positive cases by the quality of staining (strong versus weak) and by completeness (completely positive versus a fraction of the tumour cells positive). Complete TARC staining was defined as positivity in > 90% of tumour cells.

DATA ANALYSIS

Data were recorded and analysed by using SPSS version 23 (released 2015: IBM SPSS Statistics for Windows, version 23.0; IBM Corporation, Armonk, NY, USA). Pearson's χ^2 test was used to test the relationship between patient characteristics and TARC positivity. The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICS APPROVAL STATEMENT

This study was conducted in accordance with the declaration of Helsinki. The medical ethical review board of the University Medical Center Groningen approved the protocol under #RR202100080.

Results

STUDY POPULATION

A total of 383 cases were included: 155 (40.5%) lymph node needle biopsies, 170 (44.4%) lymph node excisions, 48 (12.5%) extranodal tissue needle biopsies and 10 (2.6%) extranodal tissue excisions. A

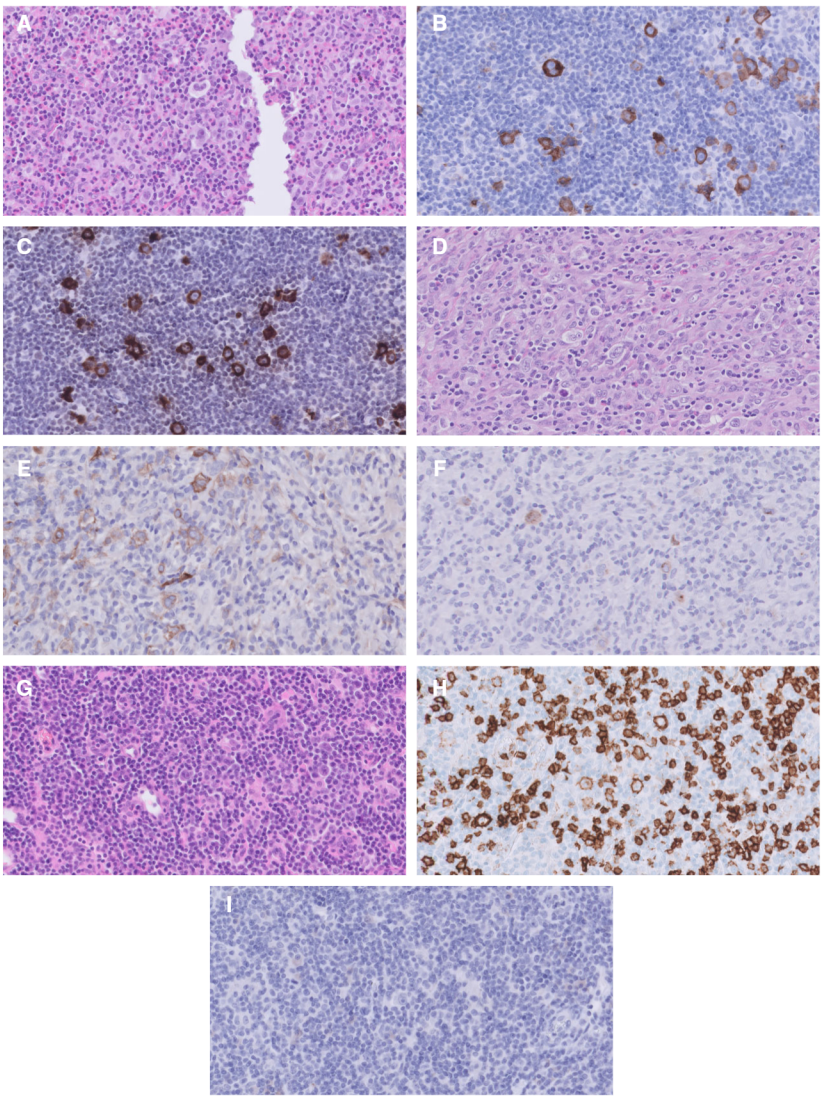

Figure 1. **A–C**, cHL with multiple HRS cells that are CD30-positive (**B**). TARC IHC in this case shows strong cytoplasmic staining in all the tumour cells (**C**). **D–F**, EBV-positive cHL with HRS cells that show diffuse CD30 positivity (**E**). TARC IHC shows a weak and incomplete staining pattern (**F**). **G–I**, Nodular lymphocyte predominant Hodgkin lymphoma with CD20-positive tumour cells (**H**). TARC IHC in this case is completely negative in all tumour cells (I). cHL, classic Hodgkin lymphoma; HRS, Hodgkin/Reed–Sternberg; IHC, immunohistochemistry; TARC, thymus and activation-related chemokine.

total of 190 cases of cHL were analysed, 20 cases of NLPHL, 64 reactive conditions and 109 cases of mature lymphoid neoplasms with (some) HRS-like cells that were at least weakly CD30-positive.

TARC STAINING IN CHL

Cytoplasmic positivity of TARC was seen in 91.6% of all patients diagnosed with cHL. The majority of cHL cases (77.4%) showed strong staining of all HRS cells. In some cases the area surrounding positive HRS cells stained weakly, with a gradient moving away from the HRS cells, probably indicating extracellular localisation of secreted TARC. Occasionally a few cells in the reactive background showed aspecific staining, but these were small and easily discernable from tumour cells. A representative example is shown in Figure 1A–C.

In 92 cHL cases (48.4%) the diagnosis was made on a lymph node needle biopsy. As a result of this high percentage of needle biopsies, many cHL cases were not subtyped. Nonetheless, lymphocyte-rich (LRCHL) and mixed cellularity cHL appeared to show less TARC staining (Table 1). Interestingly, TARC

Table 1. TARC expression on HRS cells in classic Hodgkin lymphoma and nodular lymphocyte predominant Hodgkin lymphoma

Diagnosis	Total *N*	TARC expression		
		Negative *n* (%)	Weak and/or incomplete *n* (%)	Strong and complete *n* (%)
Classic Hodgkin	190	16 (8.4)	27 (14.2)	147 (77.4)
Not subtyped	94	8 (8.5)	14 (14.9)	72 (76.6)
Nodular sclerosis	67	1 (1.5)	5 (7.5)	61 (91.0)
Lymphocyte-rich	12	4 (33.3)	2 (16.7)	6 (50.0)
Mixed cellularity	17	3 (17.6)	5 (29.4)	9 (52.9)
Sex				
Male	127	15 (11.8)	21 (16.5)	91 (71.7)
Female	63	1 (1.6)	6 (9.5)	56 (88.9)
Age category				
<45 years	127	4 (3.7)	10 (9.3)	93 (86.9)
≥45 years	63	12 (14.5)	17 (20.5)	54 (65.1)
EBV				
Negative	127	5 (3.9)	15 (11.8)	107 (84.3)
Positive	53	11 (20.8)	12 (22.6)	30 (56.6)
Nodular lymphocyte-predominant	20	18 (90)	2 (10)	0 (0)

cHL, Classic Hodgkin lymphoma; EBV, Epstein–Barr virus; TARC, thymus and activation-related chemokine.
In cHL patients, sex ($P = 0.017$), age category ($P = 0.008$) and EBV status ($P = 0.001$) were significantly related to TARC positivity (any positivity).

staining was also related to the EBV status of the tumours. Of all TARC-negative cases, 68.8% was EBV-positive. Negative, weak or incomplete TARC staining was seen in 43.4% (23 of 54) of EBV-positive cHL cases compared to 15.7% (20 of 127) in the EBV-negative cHL subgroup ($P = 0.001$). Examples of weak and incomplete TARC staining in an EBV-positive case can be seen in Figure 1D–F. In addition, patient characteristics were significantly related to TARC positivity. TARC IHC was more frequently positive in female patients compared to males ($P = 0.017$). Also, younger patients (aged < 45 years) were more likely to have positive TARC IHC ($P = 0.008$).

To gain more insight into the added value of TARC staining in the diagnosis of cHL, we compared the results to staining of CD30, CD15, PAX-5, CD20 and CD79a in tumour cells (Table 2). As expected, the majority (87.3%) of cHL cases showed strong and complete CD30 staining, while the other cases showed relatively weak expression. CD15 was strongly positive in 47.8%, weak and/or incomplete in 36.4% and completely negative in 15.7% of cases. When CD30 IHC was not strong and complete, TARC showed strong and complete staining in 12 of 24 (50%) of cases. In cases with no CD15 staining, 69% was positive for TARC. As expected, PAX-5 was the most potent indicator of B cell lineage, as 83.1% of cases showed weak positivity (compared to surrounding B lymphocytes) and 10.1% showed relatively strong and complete staining. CD20 and CD79a showed weak and/or incomplete positivity in 17.8 and 34.2% of cases, respectively. As Table 2 shows, the majority of cHL cases with B cell lineage marker expression showed strong and complete TARC staining.

Table 2. Immunohistochemical profile of classic Hodgkin lymphoma cases related to TARC expression

	Total *N*	TARC expression		
		Negative *n* (%)	Weak and/or incomplete *n* (%)	Strong and complete *n* (%)
CD15				
Negative	29	9 (31.0)	4 (13.8)	16 (55.2)
Weak/incomplete	67	6 (9.0)	15 (22.4)	46 (68.7)
Strong and complete	88	1 (1.1)	8 (9.1)	79 (89.8)
CD30				
Negative	1	0 (0.0)	0 (0.0)	1 (100)
Weak/incomplete	23	7 (30.4)	5 (21.7)	11 (47.8)
Strong and complete	166	9 (5.4)	22 (13.3)	135 (81.3)
CD20				
Negative	147	11 (7.5)	21 (14.3)	115 (78.2)
Weak/incomplete	26	2 (7.7)	5 (19.2)	19 (73.1)
Strong and complete	6	3 (50.0)	1 (16.7)	2 (33.3)
CD79a				
Negative	96	6 (6.3)	17 (17.7)	73 (76.0)
Weak/incomplete	44	7 (15.9)	4 (9.1)	33 (75.0)
Strong and complete	6	2 (25.0)	2 (25.0)	4 (50.0)
PAX-5				
Negative	12	0 (0.0)	2 (16.7)	10 (83.3)
Weak/incomplete	148	9 (6.1)	20 (13.5)	119 (80.4)
Strong and complete	18	5 (27.8)	4 (22.2)	9 (50.0)

TARC, thymus and activation-related chemokine.

TARC STAINING IN NLPHL AND LRCHL

In NLPHL (n = 20), 90% of cases showed no TARC staining of tumour cells at all, while in 10% there was only weak staining of sporadic tumour cells. There was no staining of cells in the inflammatory background. Figure 1G–I shows a representative NLPHL case. In EBV-negative LRCHL (n = 8), TARC was strongly positive in all tumour cells in six cases and this helped in the distinction with NLPHL. One challenging EBV-negative LRCHL case was TARC-negative and was preferred over NLPHL by being weakly CD30-positive, partly CD15-positive and by the lack of follicular dendritic networks, although expression of B cell markers was present. The other EBV-negative LRCHL case was weakly TARC-positive and strongly positive for both CD15 and CD30.

LACK OF TARC STAINING IN REACTIVE CONDITIONS

Lymph node biopsies with immunoblastic, large, CD30-positive cells may raise suspicion of cHL, especially in needle biopsies. In 64 cases, with 30 needle biopsies, none showed staining for TARC. Recognition as a reactive lymphadenopathy was based on the lack of classical HRS cells, low and/or heterogeneous CD30 staining, strong CD20 positivity and lack of an appropriate tumour micro-environment. Figure 2A,B shows a representative case of reactive lymphadenopathy.

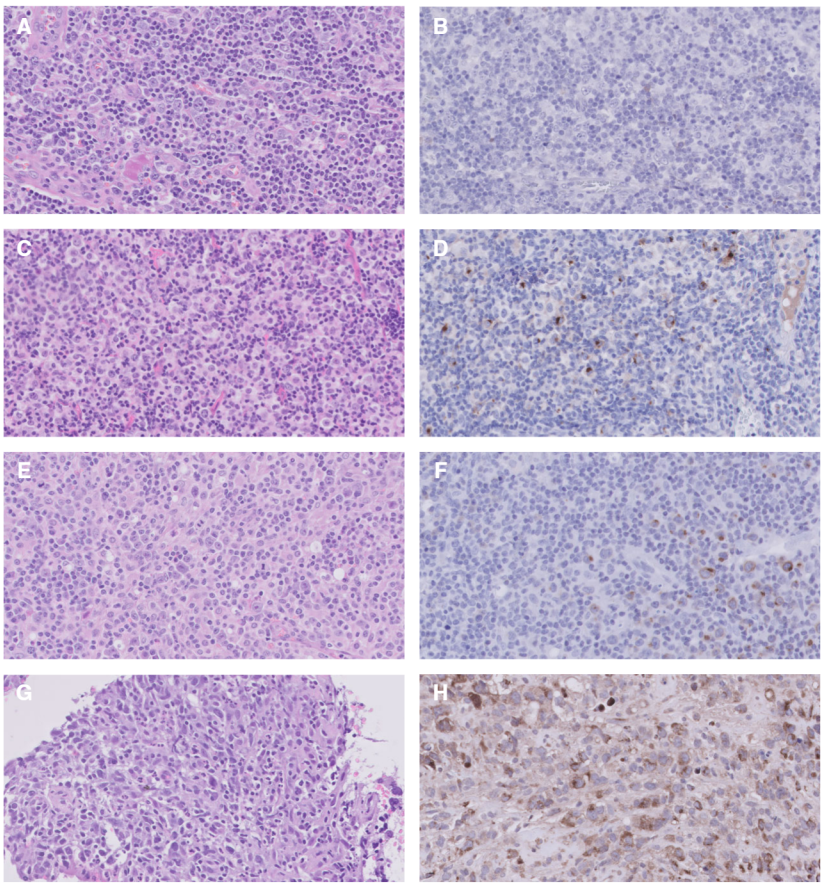

Figure 2. **A**, **B**, reactive lymphadenopathy with multiple CD30-positive immunoblastic cells. TARC proved to be completely negative (**B**). **C**, **D**, Follicular lymphoma with numerous CD30-positive large B cell blasts (**C**). TARC IHC shows variable staining in part of the blasts (**D**). **E**, **F**, B cell lymphoma unclassifiable with features intermediate between diffuse large B cell lymphoma and classic Hodgkin lymphoma. TARC IHC is weakly positive in part of the tumour cells; the remainder is negative (**F**). **G**, **H**, primary mediastinal B cell lymphoma. TARC IHC shows a weak/variable staining with clear negativity of part of the tumour cells (**H**). IHC, immunohistochemistry; TARC, thymus and activation-related chemokine.

TARC STAINING IN MATURE LYMPHOID NEOPLASMS WITH HRS-LIKE CELLS

TARC immunostaining was performed in 109 lymphoma cases with a final diagnosis other than cHL, NLPHL or reactive (Table 3). This concerned cases in which HRS-like cells with at least weak CD30 staining were present (Figure 2C–H). Strong and complete TARC staining of HRS-like cells was seen in only six cases consisting of EBV-positive diffuse large B cell lymphoma (DLBCL; two of 14), chronic lymphocytic leukaemia/small lymphocytic lymphoma with EBV-positive HRS-like blasts (one of one), peripheral T cell lymphoma not otherwise specified with EBV-positive HRS-like blasts (one of 14) and disseminated mycosis fungoides (two of two) and because most of the strongly TARC-positive mimics were EBV-positive, we also analysed the other EBV-positive mimics in more detail. In 10 T cell lymphomas that harboured a secondary EBV-positive B cell lymphoproliferation, one was strongly positive and five were weakly positive for TARC. The EBV-positive DLBCLs (nine of which were immune deficiency-related) consisted of two strongly and six weakly TARC staining cases. All these cases could be clearly separated from EBV-positive cHL by acknowledging that only a small proportion of the neoplastic cells consisted of HRS-like cells. However, TARC staining patterns on their own showed considerable overlap with those in EBV-positive cHL. Well-known cHL mimics, such as primary mediastinal B cell lymphoma and B cell lymphoma unclassifiable with features intermediate between DLBCL and cHL, frequently showed weak and/or incomplete staining (17 of 19), but none of these cases exhibited strong and complete positivity.

Table 3. TARC expression in tumour cells or Hodgkin/Reed–Sternberg-like cells in classic Hodgkin lymphoma, nodular lymphocyte-predominant Hodgkin lymphoma, mature lymphoid neoplasms and reactive/infectious/non-malignant conditions

		TARC expression		
Diagnosis	Total *N*	Negative, *n* (%)	Weak and/or incomplete *n* (%)	Strong and complete *n* (%)
Classic Hodgkin lymphoma	190	16 (8.4)	27 (14.2)	147 (77.4)
Reactive/infectious/not malignant	64	64(100)	0 (0)	0 (0)
Diffuse large/high grade B cell lymphoma NOS	29	23 (79.3)	6 (20.7)	0 (0)
Nodular lymphocyte-predominant Hodgkin lymphoma	20	18 (90)	2 (10.0)	0 (0)
EBV^+ diffuse large B cell lymphoma	14	6 (42.9)	6 (35.7)	2 (21.4)
Peripheral T cell lymphoma NOS	14	10 (71.4)	3 (21.4)	1 (7.1)
Primary mediastinal B cell lymphoma	10	2 (20)	8 (80)	0 (0)
Grey zone lymphoma[a]	9	0 (0)	9 (100)	0 (0)
Follicular lymphoma	8	1 (12.5)	7 (75)	0 (0)
Anaplastic large-cell T cell lymphoma	7	7 (100)	0 (0)	0 (0)
Angio immunoblastic T cell lymphoma	5	3 (60)	2 (40)	0 (0)
Chronic lymphocytic leukaemia	5	3 (60)	1 (20)	1 (20)
Disseminated mycosis fungoides	2	0 (0)	0 (0)	2 (100)
Marginal zone lymphoma	2	2 (100)	0 (0)	0 (0)
Other/unclassifiable lymphomas	4	2 (50)	2 (50)	0 (0)

NOS, not otherwise specified; TARC, thymus and activation related chemokine.
[a]Official name: B cell lymphoma unclassifiable with features intermediate between diffuse large B cell lymphoma and classic Hodgkin lymphoma.

Discussion

In this study we show that TARC is a highly sensitive and specific tumour cell marker for cHL and that TARC IHC can be a helpful addition in daily routine diagnostics. In our prospective setting, TARC positivity of HRS cells was seen in 92% of all cHL cases, predominantly with a strong cytoplasmic staining pattern (77%). TARC usually stains each and every HRS cell and is virtually not expressed in the tumour micro-environment, favouring its use for both screening purposes and assessment of co-expression with other tumour cell markers. This very high specificity is a clear advantage over CD30 that often stains reactive cells in the vicinity of HRS cells or in lymph nodes without cHL. It should be noted that some monoclonal TARC antibodies appear to cross-react and erroneously stain histiocytes and macrophages. We therefore employed and highly recommend a non-cross-reacting polyclonal TARC antibody.[7,9,10]

TARC IHC can help in differential diagnostic considerations in occasional challenging cases. NLPHL can be such a differential diagnosis, as it closely resembles the lymphocyte-rich subtype of cHL. The distinction can usually be made by showing that the tumour cells in NLPHL have an intact B cell phenotype with strong expression of CD20, CD79a and PAX-5, while lacking strong CD30 staining. However, HRS cells in cHL can also show variable staining of these B cell markers, and tumour cells in NLPHL are sometimes weakly CD30-positive or even CD15-positive.[13] Patterns of follicular dendritic networks detected by CD21, CD23 or CD35 IHC can help in differentiating between the two entities, but these patterns are not always present in NLPHL and can be missed in needle biopsies.[14] In our study, TARC IHC

by itself could differentiate between cHL and NLPHL in most cases, as strong and complete TARC staining was not seen in 18 of 20 NLPHL cases. The other two cases only showed weak staining of sporadic tumour cells.

In some instances, reactive lymphadenopathies may raise suspicion of cHL, especially in needle biopsies in adolescents and young adults.[15] We evaluated 64 cases in which CD30-positive cells were present that were considered potential HRS cells. These cells were usually interfollicular-reactive immunoblasts resembling relatively small mononuclear Hodgkin tumour cells. Reactive immunoblasts can show substantial CD30 positivity, albeit at lower levels than HRS cells. In all the 64 cases TARC staining was negative, and none of the biopsied individuals developed cHL in follow-up.

HRS-like tumour cells can be found in a variety of mature lymphoid neoplasms. Primary mediastinal B cell lymphoma is already known to express TARC, albeit at lower levels than in cHL.[16] Accordingly, we observed weak TARC staining in eight of 10 cases, while the other two cases were negative. B cell lymphoma unclassifiable with features intermediate between DLBCL and cHL showed weak or incomplete TARC staining in all nine cases tested, fitting well with the grey zone nature of this diagnostic category.[17] This suggests that TARC immunohistochemistry can be used to discriminate between B cell lymphomas arising from the mediastinum and other types of large B cell lymphoma. In our study, all seven anaplastic large T cell lymphoma (five ALK-negative, two ALK-positive) cases were TARC-negative, contrasting with data from two older studies that found TARC positivity in 44% of ALK negative (12 of 27) and 4% (one of 27) of anaplastic large T cell lymphomas, respectively.[5,18] We used the same antibody, but with another protocol, so this difference might be caused by technical aspects or by a chance effect on case selection.

Other mature lymphoid neoplasms are biologically more distinct from cHL and can usually be readily recognised. We included cases in our series that were clearly not cHL, but which harboured at least a few HRS-like cells. This consisted of a wide variety of entities, as described previously in many case reports and reviews.[19,20] We observed strong TARC positivity in EBV-positive DLBCL (two of 14), disseminated mycosis fungoides (two of two), peripheral T cell lymphoma not otherwise specified with EBV-positive blasts (one of 14) and chronic lymphocytic leukaemia/small lymphocytic lymphoma with EBV-positive Hodgkin-like blasts (one of five). In these cases, the presence of a concurrent cHL was ruled out by considering clinical, morphological and immunophenotypical contexts. However, especially when atypical cells are EBV-positive, TARC staining should be interpreted with some caution. We report these cases to illustrate that TARC staining, like all other IHC markers used in cHL diagnostics, should always be appreciated in context.

In conclusion, TARC IHC positivity of atypical cells, especially in a strong and complete staining pattern, has great value in differentiating between cHL, NLPHL and other lymphomas or reactive lymphadenopathies with cHL-like features. Implementation in daily diagnostics in more centres will help to delineate its discriminative potential in additional rare cHL-mimics.

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Conflicts of interest

All authors declare that there are no competing (financial) interests in relation to the work described.

References

1. Swerdlow SH, Campo E, Harris NL *et al.* eds. *WHO classification of tumours of haematopoietic and lymphoid tissues.* Revised 4th ed. Lyon: IARC, 2017.
2. Veldman J, Visser L, Huberts-Kregel M *et al.* Rosetting T cells in Hodgkin lymphoma are activated by immunological synapse components HLA class II and CD58. *Blood* 2020; **136**; 2437–2441.
3. Wang HW, Balakrishna JP, Pittaluga S, Jaffe ES. Diagnosis of Hodgkin lymphoma in the modern era. *Br. J. Haematol.* 2019; **184**; 45–59.
4. van den Berg A, Visser L, Poppema S. High expression of the CC chemokine TARC in reed-Sternberg cells. A possible explanation for the characteristic T-cell infiltrate in Hodgkin's lymphoma. *Am. J. Pathol.* 1999; **154**; 1685–1691.
5. Peh SC, Kim LH, Poppema S. TARC, a CC chemokine, is frequently expressed in classic Hodgkin's lymphoma but not in NLP Hodgkin's lymphoma, T-cell-rich B-cell lymphoma, and most cases of anaplastic large cell lymphoma. *Am. J. Surg. Pathol.* 2001; **25**; 925–929.
6. Veldman J, Rodrigues Placa J, Chong L *et al.* CD4+ T cells in classical Hodgkin lymphoma express exhaustion associated transcription factors TOX and TOX2: characterizing CD4+ T cells in Hodgkin lymphoma. *Onco. Targets Ther.* 2022; **11**; 2033433.
7. Plattel WJ, van den Berg A, Visser L *et al.* Plasma thymus and activation-regulated chemokine as an early response marker in classical Hodgkin's lymphoma. *Haematologica* 2012; **97**; 410–415.

8. Weihrauch MR, Manzke O, Beyer M *et al*. Elevated serum levels of CC thymus and activation-related chemokine (TARC) in primary Hodgkin's disease: potential for a prognostic factor. *Cancer Res.* 2005; **65**; 5516–5519.
9. Plattel WJ, Alsada ZN, van Imhoff GW, Diepstra A, van den Berg A, Visser L. Biomarkers for evaluation of treatment response in classical Hodgkin lymphoma: comparison of sGalectin-1, sCD163 and sCD30 with TARC. *Br. J. Haematol.* 2016; **175**; 868–875.
10. Plattel WJ, Visser L, Diepstra A *et al*. Interim thymus and activation regulated chemokine versus interim (18) F-fluorodeoxyglucose positron-emission tomography in classical Hodgkin lymphoma response evaluation. *Br. J. Haematol.* 2020; **190**; 40–44.
11. Jones K, Vari F, Keane C *et al*. Serum CD163 and TARC as disease response biomarkers in classical Hodgkin lymphoma. *Clin. Cancer Res.* 2013; **19**; 731–742.
12. O'Malley DP, Dogan A, Fedoriw Y, Medeiros LJ, Ok CY, Salama ME. American registry of pathology expert opinions: immunohistochemical evaluation of classic Hodgkin lymphoma. *Ann. Diagn. Pathol.* 2019; **39**; 105–110.
13. Venkataraman G, Raffeld M, Pittaluga S, Jaffe ES. CD15-expressing nodular lymphocyte-predominant Hodgkin lymphoma. *Histopathology* 2011; **58**; 803–805.
14. Fan Z, Natkunam Y, Bair E, Tibshirani R, Warnke RA. Characterization of variant patterns of nodular lymphocyte predominant hodgkin lymphoma with immunohistologic and clinical correlation. *Am. J. Surg. Pathol.* 2003; **27**; 1346–1356.
15. Faraz M, Rosado FGN. Reactive lymphadenopathies. *Clin. Lab. Med.* 2021; **41**; 433–451.
16. Vigano E, Gunawardana J, Mottok A *et al*. Somatic IL4R mutations in primary mediastinal large B-cell lymphoma lead to constitutive JAK-STAT signaling activation. *Blood* 2018; **31**; 2036–2046.
17. Poppema S, Kluiver JL, Atayar C *et al*. Report: workshop on mediastinal grey zone lymphoma. *Eur. J. Haematol. Suppl.* 2005; **66**; 45–52.
18. Vermeer MH, Dukers DF, ten Berge RL *et al*. Differential expression of thymus and activation regulated chemokine and its receptor CCR4 in nodal and cutaneous anaplastic large-cell lymphomas and Hodgkin's disease. *Mod. Pathol.* 2002; **15**; 838–844.
19. Gomez-Gelvez JC, Smith LB. Reed-Sternberg-like cells in non-Hodgkin lymphomas. *Arch. Pathol. Lab. Med.* 2015; **139**; 1205–1210.
20. Parente P, Zanelli M, Sanguedolce F, Mastracci L, Graziano P. Hodgkin reed-Sternberg-like cells in non-Hodgkin lymphoma. *Diagnostics (Basel)* 2020; **10**; 1–30.