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Original Paper

Unique morphological spectrum of lymphomas in Nijmegen breakage syndrome (NBS) patients with high frequency of consecutive lymphoma formation

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Abstract

Nijmegen breakage syndrome (NBS) is an autosomal recessive disorder characterized by microcephaly, immunodeficiency, radiation hypersensitivity, chromosomal instability and increased incidence of malignancies. In Poland 105 NBS cases showing mutations in the NBS gene (nibrin, *NBN*), have been diagnosed, ~53% of which have developed cancer, mainly (>90%) lymphoid malignancies. This study is based upon the largest reported group of NBS-associated lymphomas. The predominant lymphoma types found in these 14 NBS children were diffuse large B cell lymphoma (DLBCL) and T cell lymphoblastic lymphoma (T-LBL/ALL), all showing monoclonal Ig/TCR rearrangements. The spectrum of NBS lymphomas is completely different from sporadic paediatric lymphomas and lymphomas in other immunodeficient patients. Morphological and molecular analysis of consecutive lymphoproliferations in six NBS patients revealed two cases of true secondary lymphoma. Furthermore, 9/13 NBS patients with lymphomas analysed by split-signal FISH showed breaks in the Ig or TCR loci, several of which likely represent chromosome aberrations. The combined data would fit a model in which an *NBN* gene defect results in a higher frequency of DNA misrejoining during double-strand break (DSB) repair, thereby contributing to an increased likelihood of lymphoma formation in NBS patients.

Keywords: Nijmegen breakage syndrome; immunodeficiency; lymphoma; immunoglobulin gene rearrangement; TCR gene rearrangement; DSB repair

Introduction

Nijmegen breakage syndrome (NBS) is an inherited condition, caused by mutation of the nibrin (*NBN*) gene on chromosome 8q21 [1–3]. The NBN protein is one of the subunits of the Mre11–Rad50–NBN (M–R–N) nuclear protein complex, which has been associated with double-strand DNA break (DSB) repair in *in vitro* studies [4–6], although other studies have questioned a role in DSB repair [7,8]. NBS is characterized by chromosomal instability, cellular and humoral immunodeficiency, sensitivity to ionizing radiation, gonadal failure and predisposition to malignancies of lymphoid origin at very young age, features that are shared with ataxia-telangiectasia (AT) and partly also with ataxia-telangiectasia-like disorder (AT-LD). AT and AT-LD are caused by *ATM* and *MRE11* gene mutations, respectively [9–10]. Mutations in the genes that encode M–R–N components result in radiation sensitivity, genome instability, telomere shortening, aberrant meiosis and, perhaps most importantly, loss of S and G_1/G_2 cell cycle check point control [9–13]. Chromosomal instability in NBS results in characteristic chromosome rearrangements involving chromosomes 7 and 14, with breakpoints at the immunoglobulin (Ig) and T cell receptor (TCR) loci [14,15]. These aberrations are thought to be caused by incorrect rejoining during V(D)J recombination [16] and mostly concern Ig/TCR translocus rearrangements (our own unpublished data), as has also been shown for healthy controls and AT patients [17].

In primary immune defects that show impaired host responses, eg to EBV infections, an increased risk of lymphoid malignancy development occurs; furthermore, inherited autosomal recessive disorders such as

AT and NBS with disrupted DNA damage responses also show an increased lymphoma/leukaemia incidence [1,18–20]. The estimated increased incidence in AT is 70–250-fold, whereas NBS patients even carry a >1000-fold risk of developing lymphoma [21–24]. In AT, both B and T cell malignancies are involved, with a four- to five-fold increase in the frequency of T cell tumours [25–30]. In NBS lymphomas seem largely of B cell origin, classified as diffuse large B cell lymphoma (DLBCLs; WHO criteria) [31–33]. or as polymorphic B-NHL in immunocompromised individuals [29]. Atypical lymphoproliferations or lymphomas occurring in the setting of primary immunodeficiencies are claimed to be mostly extranodal, very aggressive, related to polyclonal activation of lymphoid cells (often driven by EBV infection) and to show specific genetic abnormalities and/or abnormal cell cycle regulation [34–36]. Here we present a unique series of immunophenotypically and molecularly characterized lymphoproliferations occurring in 14 NBS patients from the Polish NBS registry.

Materials and methods

Patients and samples

Fourteen Polish NBS Registry patients (all showing the common homozygous 657del5 *NBN* mutation) with malignant lymphoma (range 4–19 years; M : F ratio, 1 : 1.2) were the subjects of the current study (Table 1). Retrospective immunophenotypic and molecular analyses were based upon lymph node biopsies at diagnosis. In six of these NBS patients, consecutive lymphoma biopsies were analysed to evaluate their potential clonal relationship. The studies described here were performed following the Helsinki guidelines and were approved by the CMHI Institutional Review Board.

Histology and immunohistochemistry

All tissue biopsies were processed similarly, i.e. one part fixed in 10% buffered formalin and routinely processed, and the second part frozen in liquid nitrogen. Whenever necessary sections were microwave pretreated in 0.1 M citrate buffer, pH 6.0, or digested with trypsin. Immunostaining on frozen and paraffin-embedded tissue was performed using Envision (Dako Denmark A/S, Glostrup, Denmark), streptavidin or APAAP techniques. Appropriate controls (reactive tonsil, reactive lymph node, plasma cell proliferation) were included in all stainings. A WHO diagnosis was reached based on histomorphology and immunohistochemistry [35].

Genomic DNA isolation, PCR amplification and heteroduplex analysis

Genomic DNA was extracted from frozen and/or paraffin-embedded tissue biopsy material, using the GenElute Mammalian Genomic DNA kit (Sigma Genosys, Pampisford, UK). *IGH, IGK, IGL, TCRB, TCRG* and *TCRD* gene rearrangements were analysed using the BIOMED-2 multiplex PCR protocol [36]. Appropriate positive, polyclonal and negative controls were included in each assay [36]. Ig/TCR PCR products were further subjected to heteroduplex analysis [37] to discern between clonal (homoduplex bands) and polyclonal (heteroduplex smears) products. PCR amplification of translocations t(11;14) and t(14;18) and *TAL1* gene deletions (types 1 and 2) were performed as described [36,38].

Sequencing

Clonal Ig/TCR PCR products were purified from polyacrylamide gels and subjected to sequence analysis on an ABI 377 sequencer, using the dye-terminator cycle

Table 1. Histological diagnosis and Ig/TCR clonality testing in primary lymphoma samples in 14 NBS patients

Case No.	Gender; age (years) (at NBS Dx*)	Age (years) (at NHL Dx)	Anatomical presentation of lymphadenopathy/lymphoma (biopsy site)	Lymphoma diagnosis	Ig/TCR Clonality	Age at death† (years) or at last examination (follow-up in years)
1	M; 11 (months)	9	Abdominal, splenomegaly (cervical tumour)	Burkitt-like	Clonal Ig	15.5 (6.5)
2	M: 15	24	Abdominal (inguinal LN)	DLBCL	Clonal Ig	†27.5 (3.5)
3	F; 6	11.5	Generalized (axillary LN)	DLBCL	Clonal Ig	†12 (0.5)
4	F; 2	6.5	Cervical (cervical LN)	DLBCL	Clonal Ig	†7.5 (1.0)
5	F; 1.5	4	Cervical (cervical LN)	DLBCL	Clonal Ig (+ EBV)	†6 (2)
6	F; 14	15	Generalized (axillary LN)	DLBCL	Clonal Ig	†20.5 (5.5)
7	M; 11	11	Cervical (cervical LN)	DLBCL	Clonal Ig	27.5 (16.5)
8	M; 1.5	4	Generalized (cervical LN)	DLBCL	Clonal Ig (+ EBV)	†5 (1)
9	F; 1.5	8	Generalized (axillary LN)	DLBCL	Clonal Ig	†10.5 (2.5)
10	M; 5	12	Cervical (cervical LN)	cHL	Polyclonal	†14 (2)
11	F; 8	8	Generalized (cervical LN)	AILT-like B cell lymphoma	Clonal Ig	†8.5 (0.5)
12	F; 7	8	Cervical (cervical LN)	T-LBL/ALL	Clonal TCR	†11.5 (3.5)
13	M; 10.5	16	Cervical (cervical LN)	T-LBL/ALL	Clonal TCR	†18 (2)
14	M; 12	12	Cervical (cervical LN)	T-LBL/ALL	Clonal TCR	†13 (1)

* All patients showed genetic confirmation of NBS (657del5 *NBN* mutation).

sequencing kit with Ampli*Taq* FS DNA polymerase or on an ABI3100 capillary sequencer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Involved V/D/J genes were identified using the IMGT database and bioinformatics tools (http://imgt.cines.fr:8104).

Ig/TCR fluorescence *in situ* hybridization analysis (FISH)

Split-signal FISH was performed using probes for the different antigen receptor genes: IGH (14q32; Y5406), IGK (2p11;Y5416), IGL (22q11;Y5412), TCRB (7q34;Y5421), TCRG (7p14;Y5420) and TCRAD (14q11;Y5419). Probes, Histology and Cytology FISH Accessory Kits (K5599 and K5499), Hybridizer system (No. 2451) and a Whirlpool JT356 microwave were from Dako. The fluorescent microscope was equipped with double filters suitable for the FITC- and Texas red-labelled split-signal probes. Paraffin embedded, neutral-buffered formalin-fixed biopsies were used. Cases were placed in duplicate on a tissue microarray (core diameter 0.8 mm) and subjected to FISH, following standardized procedures for *in situ* hybridization (www.euro-FISH.org). All cores were evaluated by two independent observers and images were captured for documentation.

Results

Histopathology of NBS lymphomas

The diagnosis and subsequent classification of the malignant lymphomas in the 14 NBS patients (Table 1) was based on combined morphology and immunohistochemistry (see Supporting information, Table S1, for details). NBS lymphoma case 1 showed a Burkitt-like lymphoma with a diffuse pattern of medium-sized cells with round nuclei and a prominent starry-sky pattern. Eight NBS patients (cases 2–9) were diagnosed with DLBCL with an activated B cell immunophenotype (CD10- and BCL6-negative), showing predominantly immunoblasts ($n = 3$ cases), centroblasts ($n = 2$), or single, pleiomorphic, atypical multinucleated cells ($n = 2$). Proliferation marker Ki-67 expression was uniformly high in all cases. Two DLBCL cases displayed strong EBER nuclear signals in >75% of cells in conjunction with clonal episomal EBV genome, suggestive of a role for EBV in lymphomagenesis. All other DLBCLs only showed single $EBER^+$ nuclei or were EBER-negative. Attempts to stratify NBS DLBCL cases into morphological variants failed, due to the impossibility of fulfilling the strict WHO criteria of morphological variants. Cases 10 and 11 showed histopathological features of classical Hodgkin's lymphoma (cHL) of mixed cellular type and angioimmunoblastic T cell lymphoma (AILT)-like B cell lymphoma, respectively. NBS lymphomas 12–14 were classified as TdT^+ T cell lymphoblastic lymphoma/acute lymphoblastic leukaemia (T-LBL/ALL) with a very high proliferation rate (75–90%), as documented by Ki-67 staining.

Ig/TCR clonality studies in NBS lymphomas

To assess the clonal character of the histopathologically analysed lymphoproliferations of NBS patients, Ig/TCR clonality studies were performed by means of BIOMED-2 multiplex PCR heteroduplex analysis (Table 1) and sequencing (see Supporting information, Table S2). Complete in-frame V_H–J_H rearrangements were identified in the Burkitt-like lymphoma, in six of eight DLBCL cases, and in the AILT-like B cell lymphoma case. In the Burkitt-like lymphoma case and three DLBCL cases, clear signs of somatic hypermutation were observed. Clonal *IGK* PCR products were identified in several NBS lymphomas, including the two DLBCL cases in which no clonal IGH rearrangements were found; in four cases this concerned in-frame $V\kappa$–$J\kappa$ rearrangements, whereas the others contained deletional ($V\kappa$–Kde or $V\kappa$–$J\kappa$ + intron–Kde) rearrangements. In-frame *IGL* clonal PCR products were observed in three NBS lymphomas. Two T-LBL/ALL cases showed (in)complete *TCRD* rearrangements, with an additional in-frame $V\gamma$–$J\gamma$ PCR product in one. Complete $V\beta$–$J\beta$ rearrangements were identified in the third T-LBL/ALL case. No clonal TCR products were identified in the AILT-like B cell lymphoma case. Because no karyotyping data were available on the NBS cases, we attempted to further characterize the lymphomas for frequently occurring chromosome aberrations by PCR. However, no indications were found for t(11;14) or t(14;18) translocations in the B cell lymphomas, or for *SIL–TAL1* genomic microdeletions in the T-LBL/ALL cases (data not shown).

Consecutive lymphomas in NBS patients

During and after treatment of the NBS patients, consecutive lymphomas developed in six patients (cases 1, 2, 6,7, 9,10), which were compared with respect to immunohistochemistry and Ig/TCR gene status to assess the potential clonal relationship between the lymphomas (Table 2). In two cases the morphology and immunophenotypic profile were clearly different between the consecutive lymphomas: in case 1 the initial Burkitt-like lymphoma was followed by development of a DLBCL 1.5 years later, whereas in case 2 a PTCL-U developed 3 years after the DLBCL. As the clonal Ig/TCR gene rearrangements of the consecutive lymphomas appeared unrelated in both cases, the molecular data thus confirm the idea of second lymphoma rather than relapse. This is in complete contrast to cases 7 and 9, in which the initial DLBCL histopathology was preserved in consecutive lymphomas (case 7, 7 and 6 years later; case 9, 2 years later), suggestive of relapses of the same lymphoma.

Table 2. Immunohistochemical and molecular characterization of consecutive lymphomas in six NBS patients

Case No.	Immunohistochemical diagnosis initial lymphoma	Immunohistochemical diagnosis second lymphoma	Time lapse (years)	Ig/TCR clonality pattern in consecutive lymphomas	Most likely conclusion
1	Burkitt–like (CD20/BCL6/CD10 + /−)	DLBCL (CD20/BCL6^{+}/$^{-}$/CD10)	1.5	Not related	Second lymphoma
2	DLBCL (CD20/BCL6 neg/CD3 neg)	PTCL–U (CD20 neg/BCL6 neg/CD3)	3	Not related	Second lymphoma
6	DLBCL (CD20/BCL6 neg/BCL2)	DLBCL (CD20/BCL6 neg/BCL2)	5	n.e.	Morphological relapse (no molecular confirmation possible)
7*	DLBCL (CD20/BCL6 neg/BCL2 neg)	(a) DLBCL (CD20/BCL6 neg/BCL2)	7	Identical (Vλ2.18–Jλ2/3)	Relapse
		(b) DLBCL* (CD20/BCL6 neg/BCL2)	6*	Identical (Vλ2.18–Jλ2/3)	
9	DLBCL (CD20/BCL6 neg/BCL2)	DLBCL (CD20/BCL6 neg/BCL2)	2	Identical (Vλ2.11–Jλ3)	Relapse
10	cHL(–like) (CD30/CD15 + /−/EBER)	cHL(-like) (CD30/CD15^{+}/$^{-}$/EBER)	1	n.e.	Morphological relapse (no molecular confirmation possible)

* In this case two consecutive lymphomas were diagnosed.
DLBCL, diffuse large B cell lymphoma; HL-like, Hodgkin lymphoma-like; n.e., not evaluated; PTCL-U, peripheral T cell lymphoma (unspecified).

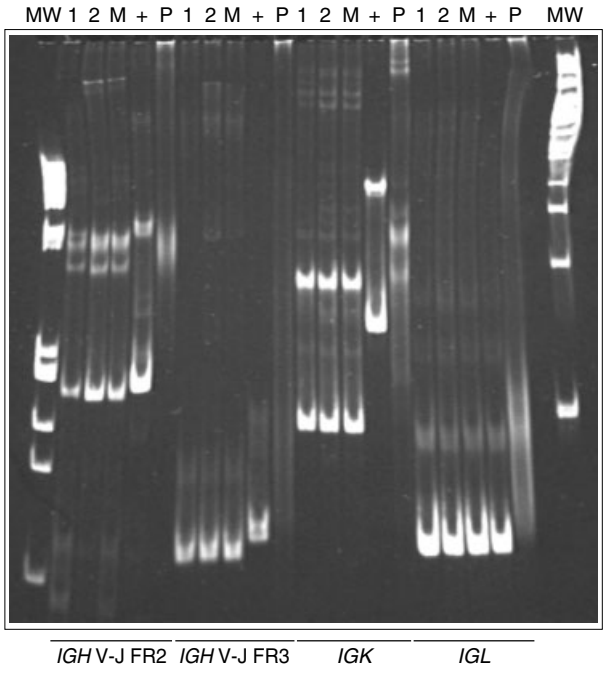

Figure 1. Clonal identity of two consecutive morphologically similar DLBCLs in an NBS patient. PCR heteroduplex analysis discloses identical clonal rearrangements of both Ig heavy and Ig light chains in primary (1) and secondary (2) tumours of DLBCL case 7, as well as in the mixed situation (M). Lanes + and P represent clonal and polyclonal controls, respectively

This assumption was confirmed by Ig/TCR clonality analysis, showing identical *IGH* and *IGK/IGL* rearrangements in the consecutive proliferations of both cases, as illustrated for case 7 in Figure 1. Finally, cases 6 (two DLBCLs, 5 years apart) and 10 (two cHL-like proliferations, 1 year apart) morphologically represented relapses as well, but due to lack of good DNA in at least one of the samples it was impossible to confirm this molecularly.

FISH-based identification of Ig and/or TCR breaks in NBS lymphomas

Given the predominance of mature B cell lymphomas and immature T cell lymphomas, which are entities known to be associated with Ig/TCR translocations, and given that the *NBN* defect is associated with genomic instability in Ig/TCR loci, we next aimed to obtain insight into the genetic aberrations associated with lymphoma formation in NBS patients. To this end, we analysed paraffin sections of the (consecutive) lymphomas by FISH, using specific probes flanking the *IGH, IGK, IGL, TCRB* and *TCRG* loci (Table 3). In six of eight available B cell lymphomas (cases 1, 3–7), breaks were observed in one or more Ig loci in the tumour cells (Figure 2). In the DLBCL lymphomas 2 and 9, no Ig aberrations were observed in either of the two consecutive lymphoma samples, whereas in patient 1 the Ig breaks were only apparent in the DLBCL lymphoma stage and not in the initial Burkitt-like lymphoma. Even though the parallel presence of breaks in both *IGH* and *IGK/IGL* loci might still represent so-called 'trans-Ig' rearrangements (ie between different Ig loci on different chromosomes), the isolated occurrence of *IGH* breaks in several DLBCLs (cases 3–6) is suggestive of pathogenic chromosome aberrations. In the AILT-like B cell lymphoma (case 11), neither Ig nor TCR breaks could be identified, whereas in two of the three T-LBL/ALL (cases 13 and 14), clear signs of TCR breaks were observed. Interestingly, T-LBL/ALL case 14, with breaks in both the *TCRB* and *TCRG* loci, showed an atypical *TCRG* southern blot band, and subsequent trans-locus PCR analysis identified clonal Vγ8–Jβ2.7 and Dβ2–Jγ1.3/2.3 trans-rearrangement products. In contrast, T-LBL/ALL case 13 showed an isolated *TCRB* break, probably representing a true *TCRB* chromosome aberration.

Table 3. Breaks in Ig and TCR loci as identified by FISH analysis in 14 NBS lymphomas

Case No.*	Lymphoma	*IGH*	*IGK*	*IGL*	*TCRB*	*TCRG*	Conclusion
1	1st	−	−	−	−	−	No aberration
	2nd	+	+	+	−	−	Ig aberration
2	1st	−	−	−	−	s	No aberration
	2nd	−	−	−	−	s	No aberration
3	1st	+	s	−	−	−	Ig aberration
4	1st	+	−	−	−	s	Ig aberration
5	1st	+	−	−	−	−	Ig aberration
6	1st	+	−	−	−	−	Ig aberration
	2nd	+	−	−	−	−	Ig aberration
7	1st	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	2nd	+	+	+	−	−	Ig aberration
	3rd	−	s	+	−	s	Ig aberration
8	1st	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
9	1st	−	−	−	−	−	No aberration
	2nd	−	−	−	−	−	No aberration
10	1st	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	2nd	−	−	−	+	+	TCR aberration
11	1st	−	−	−	−	−	No aberration
12	1st	−	−	−	−	−	No aberration
13	1st	−	−	−	+	−	TCR aberration
14	1st	s	−	−	+	+	TCR aberration

* Case numbers (incl. diagnosis) correspond to those in Tables 1 and 2.

+, Break-apart probe signals identified; −, no break-apart probe signals observed; s, sporadic cells with break-apart probe signals; n.a., not available.

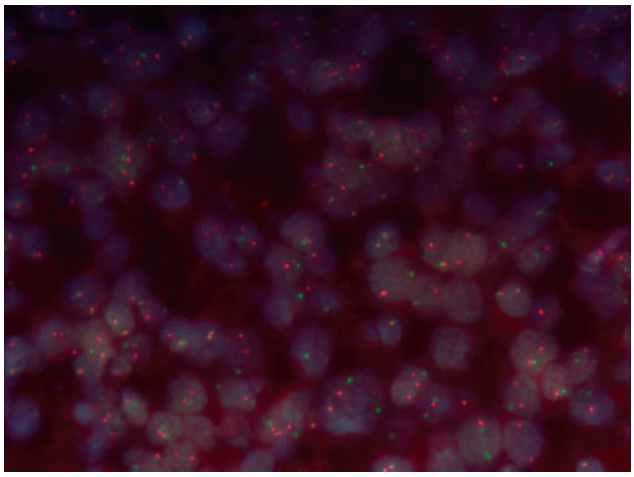

Figure 2. Immunoglobulin split-signal FISH analysis in NBS lymphoma. Split-signal FISH analysis showing breaks in *IGH* genes in paraffin-embedded tissue of DLBCL case 6. Green and red signals reflect fluorescein-labelled IGH-upstream and Texas red-labelled IGH-downstream probes, respectively. In the majority of cells separate green and red signals are observed, which illustrates a break in the human *IGH* locus; occasional cells with co-localized signals reflect non-tumour cells

Finally, cHL case 10 also showed breaks in both the *TCRB* and *TCRG* loci, which were confirmed by the presence of a clonal Dβ2–Jγ2.1 trans-rearrangement upon PCR analysis. Taken together, these data illustrate a high frequency of cases (9/13) with NBS lymphomas showing Ig/TCR breaks. In at least five cases these could represent oncogenic translocations, but this awaits final confirmation upon cloning and identification of the involved partner genes in future studies.

Discussion

The Polish NBS Registry consists of 105 patients, 56 (~53%) of whom have developed primary malignancies, including 51 cases with malignant lymphoma (as at October 2007). This is a remarkably high frequency amongst chromosome breakage syndromes in general, and the highest amongst congenital immunodeficiencies [21–25,29,30,39]. Here we present the first comprehensive study on clinical and biological aspects of the largest reported series of malignant NBS lymphomas. Our present data substantiate earlier notions [31–33] that DLBCL is one of the dominant NHL subtypes in NBS patients. The other very frequent NHL type in our cohort was T-LBL/ALL, with the remainder concerning single cases of AILT-like B cell lymphoma, classical HL and Burkitt-like lymphoma. Six NBS DLBCLs revealed BCL2 positivity, which is sometimes considered to be a poor prognostic factor [40,41]. This is in keeping with an aggressive biological behaviour, with most NBS patients dying within ~5 years following NHL diagnosis. In all our NBS DLBCLs, monoclonality was confirmed by Ig rearrangement studies. This is contrary to other primary or secondary immunodeficiencies, in which ~60% of B cell lymphoproliferations are oligoclonal and polymorphic [30]. In two NBS DLBCL cases, only clonal *IGK* rearrangements could be found by PCR, probably reflecting somatically mutated *IGH* genes. Evidence for somatic hypermutation was indeed observed in rearranged *IGH* PCR products of four others, confirming the (post-)germinal centre cell origin of NBS DLBCLs.

Table 4. Relative frequencies of NHL subtypes in NBS children as compared to NHL in individuals with other primary/secondary immunodeficiencies and sporadic paediatric NHL

Lymphoma type	NBS patients (this study)	AT patients [29,30,32,39,58–60]	Immunodeficient and immunocompromised patients [24,29,30,49,50,61–64]	Sporadic paediatric patients [43,45]
Burkitt (-like)	7% (1/14)	Single reports [30,58]	High	35–50%
Diffuse large B cell	57% (8/14)	Single reports [29,58,59]	High	<2%
Anaplastic large cell	0% (0/14)	Unknown	Unknown	20–35%*
Lymphoblastic T cell	21% (3/14)	Single reports [32]	Anecdotal	30%
AILT-like B cell	7% (1/14)	Unknown	Unknown	<2%
Classical Hodgkin	7% (1/14)	Single reports [29,39,58,60]	Moderate	30–35%

* Including large B cell NHL cases.

Collectively, our data point to very specific morphological and immunophenotypic patterns in NBS lymphomas, predominantly linked with mature large B cell and precursor T cell phenotypes. This pattern is remarkable in comparison to sporadic paediatric NHL [42–45] and to NHL in individuals in different primary or secondary immunodeficient states (Table 4). Especially, the high DLBCL incidence in NBS patients, compared to 8–12% of sporadic paediatric NHLs, is striking [44,45]. In contrast, DLBCLs account for 30–40% of adult NHL, [46,47] but typical adult-type (follicular, mantle cell or marginal zone) NHLs rarely occur in children [28,48]. The spectrum of lymphoproliferations in paediatric primary or secondary immunodeficiencies is also quite different. Although clinically and morphologically heterogeneous between immunodeficiency syndromes, these lymphoproliferations share several features, including frequent extranodal involvement, polymorphic diffuse aggressive histology, and often oligoclonality [29,30,34]. Besides, most of them present with a B cell phenotype, although in AT, a syndrome with similar molecular features to NBS, a four- to fivefold increase in frequency of T cell malignancies is observed, with young adults showing predisposition to T-PLL.

With respect to our other NBS NHL types, Burkitt-like NHL and T-LBL/ALL cases have been documented in other DSB repair syndromes [6,39]; cHL is uncommon in paediatric primary immunodeficiency (3/125 cases) [49]. In post-transplant and AIDS-immunocompromised individuals, the pathogenic mechanism underlying lymphoma development includes polyclonal activation of lymphoid cells by viruses such as EBV [50], with clonal EBV genome being found within the lymphoma cells. In our NBS lymphoma series, the presence of clonal EBV genome could be established in only two DLBCL cases, whereas three others showed EBER signals in single cells only. This suggests that EBV does not play a uniform driving role in NBS lymphoma formation.

Detailed analysis of six NBS patients showing multiple lymphoproliferations over time revealed two types of consecutive lymphomas. One type concerns true second lymphoma, as found in two of the six cases. This is a high frequency (~33%) that most probably reflects the general inherited predisposition of NBS patients to lymphoma development, resulting in patients developing lymphomas more than once in their lives. In the four other NBS patients, (partial) evidence was found for the fact that the second lymphoproliferation concerned a relapse of the initial lymphoma. In one case even a third identical proliferation was observed over a total follow-up period of ~13 years. The relatively high frequency of DLBCL relapses might be due to the relatively mild treatment regimen in NBS patients, who are radiation-sensitive and prone to develop genomic instability.

NBS belongs to the congenital immunodeficiency disorders, with impairment of B and T cell function. However, neither NBN nor any other M–R–N protein appears to have a direct role in V(D)J recombination or to be fundamentally required for the generation of a diverse B cell repertoire [10,36,51]. Rather, a role for NBN and also ATM has been hypothesized in DSB DNA repair processes, whereas the overlap between clinical and cellular phenotypes in NBS and AT suggests that NBN and ATM may function through similar pathways [52–54]. DSB are physiologically created during V(D)J recombination in B and T lymphocytes and also during somatic hypermutation and class switch recombination processes in B lymphocytes [19,55]. Genetic instability of Ig/TCR genes during lymphoid development is known to result in the formation of lymphoid-specific oncogenic chromosome translocations involving Ig/TCR loci. In this respect it is noteworthy that the two predominant types of NBS lymphomas, ie mature DLBCL and immature T-LBL/ALL, represent lymphomas that are renowned for harbouring such Ig and TCR gene translocations, respectively [56,57]. Next to that, Ig or TCR locus breaks were identified in 9/13 available NBS lymphomas, at least five of which could represent true oncogenic translocations. We would therefore like to hypothesize that in NBS patients DSB misrejoining [16] through *NBN* gene disruption results in the formation of Ig/TCR translocations, which are implicated in NBS lymphomagenesis.

In conclusion, we present a unique series of NBS-associated lymphomas from the Polish NBS Registry, which were characterized immunohistochemically and molecularly. The picture emerging is that these monoclonal lymphoproliferations predominantly

show mature large B cell and precursor T cell phenotypes, which is a quite different spectrum from that of sporadic paediatric NHL and NHL in immunodeficient patients. This may reflect a different mechanism of lymphomagenesis in NBS patients, in which NBN dysfunction through a higher frequency of DNA misrejoining contributes to an increased likelihood of chromosome aberrations and subsequent lymphoma formation. This concept is strengthened by the high frequency of breaks in Ig/TCR loci in NBS lymphomas, several of which likely represent true chromosome aberrations. Further karyotypic and molecular genetic analysis is needed to identify these Ig/TCR chromosome aberrations and to confirm their recurrence in new NBS lymphoma cases. Finally, in six NBS patients development of multiple lymphomas was observed, which concerned four relapses of the initial lymphoma and two cases with true second lymphomas. The unusual occurrence of second NBS lymphomas in two of six cases is probably caused by the NBS predisposition to lymphoma development, together with the mild treatment regimen in view of the risk of genomic instability.

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Supporting information

Supporting information may be found in the online version of this article.

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