Association of aplastic anaemia and lymphoma: a report from the severe aplastic anaemia working party of the European Society of Blood and Bone Marrow Transplantation

Although anecdotal reports showing the uncommon association of aplastic anaemia (AA) and lymphoma have been published (Dorr *et al*, 1996; Koziner *et al*, 1975; Medinger *et al*, 2012; Suzuki *et al*, 2009; Veidt *et al*, 2005; Yoshioka *et al*, 1999; Zonder *et al*, 2002), data related to its incidence, diagnostic characteristics and centre management strategies are lacking. The Severe Aplastic Anaemia Working Party of the European Society of Blood and Bone Marrow Transplantation (SAAWP-EBMT) aimed to evaluate the diseases characteristics and treating centre attitudes regarding the treatment and outcome of patients suffering from both AA and lymphoma. Between 2013–2015, we collected data of patients with this combination. Congenital bone marrow failures, as well as AA or lymphoma that occurred after haematopoietic stem cell transplantation (HSCT) were excluded.

Eighty-three (28%) of the 294 EBMT centres contacted participated in this study; 13 (16%) centres, from 7 different countries, reported the 26 cases included in the study. AA was diagnosed between 1983–2015; median age at AA diagnosis was 57 (10–78) years, 16 patients (62%) were male, 13 patients had severe AA (SAA) and 6 very severe AA (vSAA). Of the 18 cases investigated for paroxysmal nocturnal haemoglobinuria (PNH) clones by flow cytometry, two had a small clone, without clinical PNH signs or symptoms. Cytogenetics was available in 22/26(85%) cases; only one had an anomaly (trisomy 12). At AA diagnosis, 85% and 86% of patients required red blood cell and platelet transfusions, respectively. Lymphoma was diagnosed between 1958 and 2015, at a median age of 52 years (12–78). Five patients (19%) had Hodgkin lymphoma (HL) for which histopathology was available in 4 cases: 2 lymphocyte predominant type, 1 nodular sclerosis and 1 mixed cellularity. Non-Hodgkin lymphoma (NHL) was reported in 21 (81%) patients: 19 (73%) had a B-cell NHL and two patients an unspecified lymphoma. A subtype of B-cell NHL was reported in 18 cases: 4 diffuse large B-cell, 3 lymphoplasmacytic, 3 follicular, 3 nodal marginal zone, 2 chronic lymphocytic leukaemia, 1 splenic marginal zone, 1 mantle cell and 1 plasma cell neoplasm. The lymphoma was treated in 23 cases (91%).

Lymphoma was detected at three different times with respect to AA (Fig 1): 11 patients presented with lymphoma before AA, 7 patients were simultaneously diagnosed with AA and lymphoma, and 8 patients presented with lymphoma after AA diagnosis .

Patients presenting AA and lymphoma simultaneously were significantly older compared to the other times of presentation (Table I). HL was mainly observed in patients presenting lymphoma before AA (4 out of 5 HL). Various underlying pathogenic mechanisms may be involved in the different times of presentation. AA occurring after lymphoma and/or its treatment could have a different cause of marrow failure than typical AA. Alkylating agents included in lymphoma therapy, could lead to exhaustion of the stem cell pool, resulting in subsequent marrow failure (Gobbi *et al*, 2009). In line with this hypothesis, we found that more patients with AA occurring after lymphoma failed to respond to standard immunosuppressive therapy (IST) and needed a HSCT (Table I). Likewise, the use of purine nucleoside analogues have significant cytotoxic activity, resulting in prolonged lymphocyte depletion, especially in CD4 T-cells; this immune dysregulation might facilitate autoimmunity. In our series, only one patient was treated with fludarabine before the development of AA. When AA and lymphoma occur concomitantly, AA may emerge as a paraneoplastic autoimmune phenomenon of the lymphoma (Chandor, 1988), although our data could not fully confirm this hypothesis. In 3 of the 7 cases with concomitant presentation, the lymphoma was treated first, aiming to control both diseases; however, after chemotherapy, complete remission of the lymphoma was achieved in 2 of these cases but the AA did not respond and needed subsequently therapy. Furthermore, lymphoma has been reported following administration of anti-thymocyte globulin (ATG) (Calistri *et al*, 2006). In this series, lymphoma was diagnosed in 2 patients within one year after ATG therapy; in both cases, the AA remained active at lymphoma diagnosis (1 partial response, 1 non-responder). In this study, none of the reported lymphomas were Epstein–Barr virus associated. All these pathophysiological hypotheses concerning the association of AA and lymphoma are conceivable, but need to be proven.

In patients where AA appeared first, the standard AA approach for therapy was observed, thus all patients received

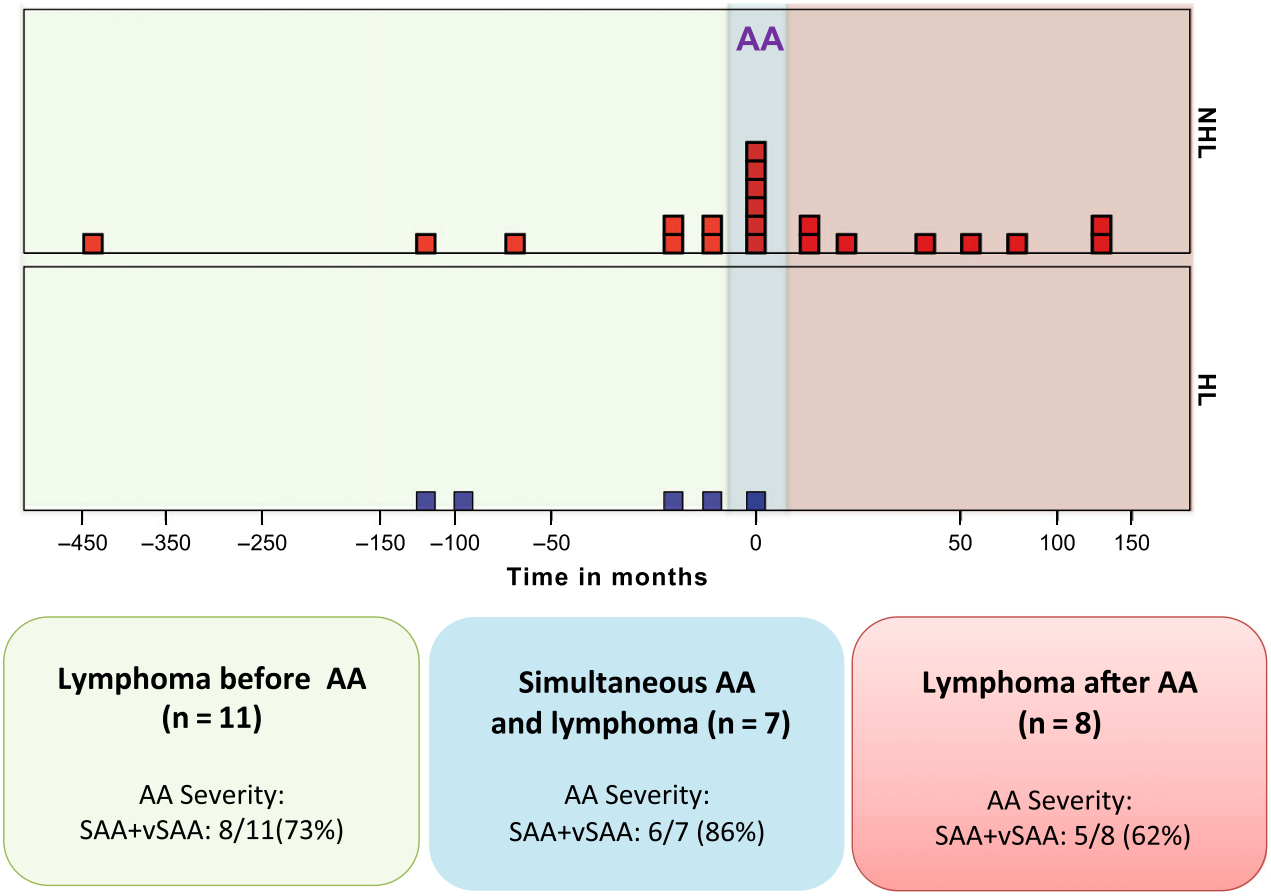

Fig 1. Order of lymphoma appearance with regard to aplastic anaemia. AA, aplastic anaemia; HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphoma; SAA, severe aplastic anaemia; vSAA, very severe aplastic anaemia.

ATG. The absence of HSCT as first line treatment is explained by the exclusion criteria of this study. The management of AA was more heterogeneous when both diseases appeared simultaneously or when AA occurred after lymphoma. When presentation was concomitant, although 6 of the 7 patients had SAA or vSAA, only 1 patient received a standard IST including ATG. In 3 cases the lymphoma was treated first, and 2 cases received treatment including other drugs, such as alemtuzumab and steroids, aiming to simultaneously treat both diseases. The centres reported that therapy was oriented to treat the more severe, or symptomatic disease. When AA occurred after the lymphoma, the management approach was also heterogeneous, including one patient who received HSCT as first line therapy.

Patients' outcome analysis showed that 17 (65%) patients were alive at last follow-up. The 9 deaths included 6 due to AA (either from the disease itself or HSCT-related cause), 1 after HSCT to treat the lymphoma; 1 from a second malignancy and 1 due to advanced age. AA-related causes of deaths were significantly more frequent than lymphoma-related deaths ($P < 0{\cdot}001$).

The presented data do not enable clear recommendations to be made regarding the management of AA when associated with lymphoma. However, centre preferences can be discussed. When AA presents first, it is clear that AA has to be treated according to its severity following the standard of care. The appearance of lymphoma later in the course does not seem to affect the prognosis. The decision to treat AA is more controversial when lymphoma appears before or at the same time as AA. Given that the outcome of AA seems to prevail over that of lymphoma, patients should receive, whenever possible, standard treatment for AA. Given there is a probability of stem cell exhaustion after lymphoma treatment (such cases will never respond to IST), an allogeneic HSCT should be considered and, if indicated, a search of a stem cell donor initiated.

In conclusion, the combination of AA and lymphoma is a rare and heterogeneous event, in which the outcome is mainly affected by the AA rather than the lymphoma. We believe that continuing systematic evaluation of such rare presentations, together with the recent achievement of molecular aspects of both diseases, might contribute to the

Table I. Association of AA and lymphoma. Comparison of disease presentation, treatment and outcome.

Parameters	Lymphoma before AA	Simultaneous Lymphoma and AA	Lymphoma after AA	*P*
N	11	7	9	
Males	7 (64%)	5 (71%)	4 (50%)	ns
Age at AA, years, (range)	49 (20–73)	68 (47–78)	41 (9–67)	0·02
Age at lymphoma, years (range)	46 (12–71)	68 (47–78)	47 (14–70)	0·02
Age at 1st diagnosis, years (range)	49 (12–73)	68 (47–78)	41 (9–67)	0·01
Median interval 1st–2nd diagnosis, in months	15 (3–436)	na	45 (4–135)	
AA Severity				
vSAA	3	1	2	
SAA	5	5	3	ns
Non-severe AA	3	1	1	
Unknown	1	0	2	
Type of lymphoma				
B-cell lymphoma	7	6	6	
HL	4	1	0	0·05
Unspecified lymphoma	0	0	2	
Treatment				
AA Therapy				
ATG containing	4	1	8	
CSA alone	5	2	0	
G-CSF	1	0	0	0·005
Alemtuzumab	0	1	0	
HSCT	1	0	0	
No treatment	0	3	0	
Response to IST				
Complete response	2	1	3	
Partial response	1	1	3	
No response	8	1	2	
Unknown	0	1	0	
First line therapy of lymphoma				
CHOP-line/HL treatment	6	2	3	
Rituximab	2	2	3	
Steroid alone	1	1	0	ns
Radiotherapy	1	0	0	
No treatment	0	2	0	
unknown	1	0	2	
Lymphoma response after therapy				
Complete remission	6	2	2	
Partial remission	1	0	3	
No response	2	3	2	
Unknown	2	0	1	
All HSCT	6 (54%)	2 (29%)	3 (31%)	ns
Indicated for AA	6	1	0	0·01
Indicated for lymphoma	0	1	3	
Outcome				
Alive	6 (55%)	5 (71%)	6 (75%)	
Death	5 (45%)	2 (29%)	2 (25%)	
Causes of death				
AA and/or its treatment	3	2	1	
Lymphoma and/or its treatment	0	0	1	
Others	2	0	0	

AA, aplastic anaemia; ATG, anti-thymocyte globulin; CHOP, cytoxan, adriamycin, vincristine and prednisone; CSA, ciclosporin; G-CSF, granulocyte colony-stimulating factor; HL, Hodgkin lymphoma; HSCT, haematopoietic stem cell transplantation;SAA, severe aplastic anaemia; vSAA, very severe aplastic anaemia.

understanding and better management of these patients in the future.

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Authorship contributions

AR served as the principal investigator for this study; AR; RPdeL; AT; ARis; JP; JM and CD contributed to the study design. AR; AK; MM; PC; JMR; RPdeL; CK; EK; BB; SM; PQ; MBCK; HS; IWB; AT; AH; ARis; JP; JM; PD and CD contributed to the data collection. AR; CK; SI; AT and CD contributed to data and statistical analysis. AR wrote the paper, AK; MM; PC; JMR; RPdeL; CK; SI; EK; BB; SM; PQ; MBCK; HS; IWB; AT; AH; ARis; JP; JM; PD and CD revised and agreed with this manuscript.

Disclosure of conflicts of interest

The authors have no conflicts of interest to disclose.

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Venetoclax as a single agent and in combination with PI3K-MTOR1/2 kinase inhibitors against ibrutinib sensitive and resistant mantle cell lymphoma

The Bruton tyrosine kinase inhibitor, ibrutinib, has shown high response rates in mantle cell lymphoma (MCL); however, many ibrutinib-treated MCL patients relapse with resistance and fulminant progression. Recently, we modelled acquired ibrutinib resistance (IR) by generating ibrutinib resistant MCL cell lines (Zhao *et al*, 2017). Using chemical proteomics and cell-based drug screen assay for drug sensitivity and response, we determined the key kinase signalling associated with IR development and revealed that adaptive kinome reprogramming contributes to increased proliferation and IR. Among the global kinase changes, sustained PI3K-AKT-MTOR pathway activation was shown to be a central hub for kinome signalling and a major determinant for cell survival, proliferation and interaction of MCL cells with tumour microenvironment (TME) stromal cells in IR MCL (Shain & Tao, 2014). These results warranted the design of mechanism-driven PI3K-MTOR inhibition-based combination therapies against MCL.

The BCL2 family proteins mediate an intrinsic, mitochondrial apoptosis pathway. BCL2, BCL-xL (also termed BCL2L1), and MCL1 are antiapoptotic BCL2 family proteins (Davids & Letai, 2012). These proteins suppress apoptosis by sequestering the BH3-only protein BIM (BCL2L11), which activates mitochondrial outer membrane permeabilization by the multi-domain pro-apoptotic proteins BAK (BAK1) and BAX. Previous studies demonstrated that BCL2 protein is highly expressed in MCL, confers MCL chemotherapy resistance, and represents an attractive therapeutic target for MCL (Campo & Rule, 2015; Sun *et al*, 2015). Recently, BH3 mimetics, such as ABT-263, which displaces pro-apoptotic proteins from BCL2 and BCL-xL and induces apoptosis in a BAX- or BAK-dependent manner, has demonstrated anti-tumour activity in B-cell malignancies but its clinical development was limited by the severe thrombocytopenia caused by BCL-xL inhibition. To avoid this toxicity, a small molecule ABT-199 (Venetoclax), a highly potent, orally bioavailable BCL2 specific inhibitor was developed and thus far is being clinically vetted as an effective therapy for many B-cell lymphomas, including chronic lymphocytic leukaemia (Vogler *et al*, 2013), multiple myeloma (Touzeau *et al*, 2014), and other lymphomas including MCL (Souers *et al*, 2013). Given these promising anti-tumour results for ABT-199, we sought to establish the extent of BCL2 expression dependency in IR MCL and evaluated the therapeutic potential of ABT-199 in IR MCL.

We interrogated ABT-199 drug response using a CCK8 based cell viability assay, mitochondrial apoptosis priming by BH3 profiling, and BCL2 protein expression levels by Western blotting in 14 MCL cell lines including parental and 3 IR MCL lines (Ryan & Letai, 2013; Zhao *et al*, 2017), and determined their correlations. Given that BH3 profiling is a functional approach used to measure the response of mitochondria to perturbation by a panel of BH3 domain peptides, thus predicting cellular response to agents that target these individual proteins (such as ABT-199), we concluded that most of MCL lines are ABT-199 sensitive with a 50% inhibitory concentration (IC50) in the nM range and associated with mitochondrial apoptosis (BH3) priming and BCL2 expression (Fig 1A–D). Intriguingly, when compared with

Fig 1. (A) Assessment of cell viability with CCK8 after ABT-199 (venetoclax) treatment for 72 h at five doses in mantle cell lymphoma (MCL) lines. 50% inhibitory concentrations (IC50s) were calculated using GraphPad Prism software (GraphPad software, Inc., La Jolla, CA, USA). (B) Correlation analysis of BCL2 protein expression levels with ABT-199 IC50. IC50s were calculated from the CCK8 assay and protein expression was measured by Western blot. (C) BH3 profiling of MCL cell lines. Mean + SD. BAD-HRK is indicative of BCL2 dependency, thus sensitivity to ABT-199 and MS-1 is indicative of MCL1 dependency. (D) Correlation analysis of ABT-199 IC50 with BCL2 (BAD-HRK) or MCL1 (MS-1) dependency. (E) BH3 profiling and ABT-199 IC50 from groups of parental MCL lines (Jeko-1, HBL-2, SP-49), acquired ibrutinib resistant (IR) MCL lines (Jeko-IR, HBL-2-IR, SP-49-IR) and *de-novo* IR lines (Maver-1, Granta-519). (F) Western blots showing the up-regulation of BCL2 and down-regulation of MCL1 in IR MCL lines when compared with parental MCL lines. Two other MCL lines (Maver-1 and Granta-519), designated as "*de novo*" IR MCL lines, were also included. (G) Cell viability and BH3 profiling assays on primary MCL samples showing primary IR MCL samples ($n = 6$) are more dependent on BCL2, and are sensitive to ABT-199 compared with ibrutinib sensitive (Sen) samples ($n = 4$).