Aplastic anemia related to thymoma: a survey on behalf of the French reference center of aplastic anemia and a review of the literature

Thymoma is associated with many autoimmune phenomena notably myasthenia gravis (MG, 45%)[1] or pure red cell aplasia (PRCA, 5%).[2] The association between thymoma and aplastic anemia (AA) is rarely reported (0-1.4% of thymoma cases).[2,3]

We conducted a retrospective study, on behalf of the French reference center (FRC) for AA, to evaluate this association in terms of risk factors, management and prognosis of patients. We investigated French patients who were diagnosed with thymoma-associated AA (T-AA) by a nationwide survey conducted by the FRC for AA. Clinical, surgical and biological data at time of thymoma and AA diagnosis were retrospectively retrieved from medical charts. Diagnosis and severity of AA were based on the criteria proposed by Camitta[4] and updated by Bacigalupo.[5] The study was performed in accordance with the Declaration of Helsinki. The institutional review board of the FRC for AA approved the study. Patients with a diagnosis of AA confirmed by a bone marrow (BM) biopsy and a history of thymoma diagnosis were included. All cases from the literature were reviewed and confirmed by expert hematologists (RPL and FSF) and pathologist (AG) who established respectively final clinical AA diagnosis and thymic diagnosis. When available data were sufficient, thymic histology and staging were established according to the 2015 World Health Organization[6] classification and the Masaoka-Koga stage classification.[7] Hematologic improvements were assessed using the British Committee for Standards in Hematology response criteria[8] (*Online Supplementary Materials and Methods*).

We report nine cases diagnosed with T-AA in France between February 2005 and July 2018 with a median follow-up time of 20.7 months (range: 1.0-157.0). At AA diagnosis, the median age was 66 years (range: 44-70) and 66.6% were women. Clinical and biological characteristics at diagnosis are reported in Table 1. Among

Table 1. Present series: patients' characteristics, aplastic anemia, thymoma presentation, treatment, response and evolution.

Pt n°	Sex/Age at AA diagnosis	AA Severity	Type of thymoma and stage*, time between AA diagnosis and thymoma resection	Good's syndrome	PRCA, time between PRCA and AA diagnosis	First line treatment and response	Second line treatment and response	Third line treatment and response	Outcome, time from diagnosis to last FU and cause of death
1	F/68	NS	AB stage I, 0.7 months after AA diagnosis	Yes	Yes, 1.3 months	Rituximab, NR	CsA+ eltrombopag, PR	Csa+eltrombopag+ everolimus, PR	Alive, 2.1 years
2	F/52	VS	B2 stage IIb, 0.5 months	No	No	ATG+CsA, NR	Danatrol+ romiplostim, NR	Rituximab+MMF, NR	Deceased, 1.4 years , severe sepsis
3	M/64	S	AB stage I, 12.1 months	Yes	Yes, 3.5 months	CSA+eltrombopag, PR	–	–	Deceased, 9.5 months, severe sepsis
4	M/68	NS	AB stage IIb, 3.1 months after AA diagnosis	No	No	CSA+eltrombopag, CR	–	–	Alive, 3.5 years
5	F/63	S	AB stage I, 1.5 months	Yes	Yes, 3.5 months	ATG+CsA, CR NR	ATG+CsA, eltrombopag	Danatrol+ 1.8 years, NR	Deceased, severe sepsis
6	F/67	S	B1 stage IIa, 18.6 months	No	No	ATG+CsA, NR	Danatrol+ romiplostim, NR	–	Deceased, 11.9 months, severe sepsis
7	F/66	NS	B1 stage II, 2.0 months	No	No	CsA, NR	CsA+danatrol, NR	ATG, NA	Deceased, 2.1 years,
8	M/44	NS	B2 stage I, 2.8 months	No	Yes, 2.0 months	CsA, NR	HSCT (brother), CR	–	Alive, 13.1 months
9	F/70	NS	B2/B1 stage IVa, recurrent thymoma 5 years after initial surgery, 4.4 months after AA diagnosis	No	No	Eltrombopag, NR	–	–	Alive, 6.3 months

*Thymic histology and staging according to the World Health Organization classification6 and the Masaoka-Koga stage classification.[7] F: female; M: male; AA: aplastic anemia; NS: non-severe; VS: very severe; S: severe; PRCA: pure red cell anemia; FU: follow-up; CsA: cyclosporine A; ATG: antithymocyte globulin; HSC: hematopoietic stem cell transplantation; NR: non-response; PR: partial response; CR: complete response; MMF: mycophenolate mofetil; NA: non-available.

Table 2. Present series and cases reported in the literature since the 1980s: patients and aplastic anemia characteristics at diagnosis.

	Included patients, N=35
Male (%)	20 (57.1)
Age at AA diagnosis in years, median (range)	57 (12-75)
AA severity (CAMITTA)	
Non-severe (%)	6 (17.1)
Severe (%)	19 (54.3)
Very severe (%)	9 (25.7)
Missing data (%)	1 (2.9)
Neutrophils, absolute count x10^9/L, median (range)	0.56 (0.00-3.70)
Hemoglobin g/L, median (range)	79 (44-121)
Lymphocytes, absolute count x10^9/L, median (range)	2.9 (0.5-8.9)
Reticulocytes, absolute count x10^9/L, median (range)	5 (0-19)
Platelets, absolute count x10^9/L, median (range)	13 (2-110)
Thymic histology (WHO classification)	
Thymoma A (%)	6 (17.1)
Thymoma AB (%)	8 (22.9)
Thymoma B1 (%)*	6 (17.1)
Thymoma B2 (%)*	9 (25.7)
Thymic carcinoma (%)	1 (2.9)
Missing data (%)	6 (17.1)
Thymic stage (Masaoka-Koga classification)	
Stage I (%)	7 (20.0)
Stage II (%)	1 (2.9)
Stage IIa (%)	1 (2.9)
Stage IIb (%)	3 (8.6)
Stage III (%)	3 (8.6)
Stage IVa (%)	3 (8.6)
Stage IVb (%)	1 (2.9)
Missing data (%)	16 (45.7)
Associated thymoma-related auto-immune disease	
Myasthenia gravis (%)	4 (11.4)
Immune thrombocytopenia (%)	3 (8.6)
Acquired autoimmune hemolytic anemia (%)	1 (2.9)
Coeliac disease (%)	1 (2.9)
Previous history of PRCA, Positive/evaluable patients (%)	7/33 (21.2)
Thymoma evolution	
Thymic resection (%)	31 (88.6)
Remission (%)	31 (88.6)
Progression (%)	4 (11.4)
Recurrence (%)	1 (2.9)
Time from thymoma resection and AA diagnosis in months, median (range)	2.8 (-72.0 - 48.0)
Disease history and transfusion	
2 courses or more previous IST (%)	14 (40.0)
>20 red blood cell units infused, positive/evaluable patients (%)	8/14 (57.1)
>20 platelet unit transfusions, positive/evaluable patients (%)	4/13 (30.8)
Death (%), positive/evaluable patients (%)	9/34 (26.5)
Causes of death	
Severe infections (%)	5 (55.6)
Pulmonary hemorrhage (%)	2 (22.2)
Thymoma progression (%)	1 (11.1)
Missing data (%)	1 (11.1)

*One patient had histology of two distinct types B1 and B2 respectively. AA: aplastic anemia; WHO: World Health Organization; PRCA: pure red cell anemia; IST: immunosuppressive therapy.

them, five (55.5%) had non-severe AA, three (33.3%) severe AA and one had very severe AA. Most patients (5 of 9) were diagnosed with AA and thymoma in a time period of no more than 2.5 months but in four patients there was a delay between thymoma resection and development of AA (range: -1.0-59.0 months). Thymectomy was performed in nine patients with thymoma remission for eight patients. In patient #9, AA was diagnosed at the onset of thymoma recurrence.

Those nine French identified cases and 26 analyzable cases of T-AA in the literature (*Online Supplementary Materials and Methods and Online Supplementary Figure S1*) were analyzed and composed a total group of 35 patients included for this study with a median observational time of 18.5 months (range: 0.25-157.0). At AA diagnosis, the median age was 57 years (range: 12-75) and 57.1% were men. The clinical and biological characteristics of the patients at AA diagnosis are reported in Table 2. Among them, six (17.1 %) had non-severe AA, 19 (54.3 %) severe AA and nine (25.7%) had very severe AA. Hematologic and immunologic characteristics of the patients are reported in Table 3. Marrow karyotype was normal in the 12 patients tested. Flow cytometry analysis revealed that 80.0% and 62.5% of evaluated patients had an inversion of CD4/CD8 ratio in the blood and BM, respectively. Hypogammaglobulinemia was observed in 7 (36.8%) of 19 evaluable patients and Good syndrome (GS) in three patients. A minor paroxysmal nocturnal hemoglobinuria (PNH) clone (<1%) was found in 3 (16.7%) of the 18 evaluable cases. Among the patients with positive acetylcholine receptor antibodies, only one had negative results at electromyography.

Thymoma histology was type A in six cases (17.1%), type AB in eight (22.9%), type B1 in six (17.1%), type B2 in nine cases (25.7%), one case of thymic carcinoma and six cases (17.1%) of missing data. Thymoma stage was heterogeneous with seven cases (20.0%) stage I, one case (2.9%) stage II, one case (2.9%) stage IIa, three cases (8.6%) stage IIb, three cases (8.6%) stage III, three cases (8.6%) stage IVa, one case (2.9%) stage IVb and 16 cases (45.7%) of missing data.

Among the entire cohort, patients developed other thymoma-associated auto-immune diseases: four (11.4%) presented with MG, three (8.6%) with immune thrombocytopenia, one (2.9%) with coeliac disease and one (2.9%) presented with acquired autoimmune hemolytic anemia. Moreover, among 33 evaluable patients, seven (21.2%) had a previous history of thymoma-associated PRCA before the diagnosis of AA within a median time of 3.5 months (range: 1.0-12.0).

Thymectomy was performed in 31 (88.6%) with thymoma remission in 30 (96.8%) patients but thymoma progression in the four non-operable patients. None of the patients had a hematologic response after thymectomy. One patient experienced thymoma recurrence 5 years after surgery at AA onset. Patients were diagnosed with a median delay of 2.8 months (range: -72.0-48.0) from thymoma resection to AA diagnosis. Twenty-four patients were thymectomized before the onset of AA, with the AA developing at a median of 4 months (range: 0.2-48.0) after surgery. Seven patients underwent surgery at the onset and/or after the diagnosis of AA.

Patient's treatment and hematologic response are reported in Table 1 and in the *Online Supplemental Table S3*. Among the entire cohort, 34 patients received 57 treatment lines (*Online Supplementary Figure S2*). The overall response rate (ORR) was 72.7% with cyclosporine A (CsA) plus antithymocyte globulin (ATG), 76.9% with CsA alone and 100% with hematopoietic stem cell transplantation (HSCT).

Table 3. Present series and cases reported in the literature since the 1980s: hematologic and immunologic characteristics.

	Positive/evaluable patients (%)
Bone marrow examination	
Hypoplasia	35/35 (100.0)
Absence of BM dysplasia	16/16 (100.0)
Normal BM cytogenetic analysis	12/12 (100.0)
CD4/CD8 ratio inversion	5/8 (62.5)
Blood examination	
Reduced B-lymphocyte cell count	6/12 (50.0)
CD4/CD8 ratio inversion	12/15 (80.0)
Hypogammaglobulinemia	7/19 (36.8)
Good syndrome	3/18 (16.7)
PNH granulocyte clone	3/18 (16.7)
PNH clone level (%), median (range)	0.05 (0.04 -0.10)
Increased or normal serum EPO level	5/5 (100.0)
low TSH level	0/7 (0.0)
Biological autoimmunity	
Anti-AchR	4/11 (36.4)
ANA	5/16 (31.3)
anti-dsDNA	2/8 (25.0)
ANCA	0/4 (0.0)
Rheumatoid factor	0/3 (0.0)
Anti-ENA	0/6 (0.0)
Anti-SSA/Ro	0/4 (0.0)
Anti-Sm	0/2 (0.0)
Anti-Jo-1	0/2 (0.0)
Anti-Mi-2	1/1 (100.0)

BM: bone marrow; PNH: paroxysmal nocturnal hemoglobinuria; EPO: erythropoietin; TSH: thyroid stimulating hormone; ANA: antinuclear antibodies; ANCA: antineutrophil cytoplasmic antibodies.

At the last follow-up, among treated patient, 10 (33.3%) patients were still in complete response (CR) and eight (26.7%) in partial response (PR). There were nine (26.5%) deaths among the 34 analyzable patients and the median overall survival was not reached at a median follow-up of 18 months (*Online Supplementary Figure S3*). The major cause of death was severe infections (55.5%). There were no significant differences in terms of survival in patients according to the thymic histology or history of PRCA, PNH clone or GS.

Our cases analysis indicate that T-AA seems more frequent in older patients than the average age of AA patients and appeared more prevalent but not statistically significant in men.[9] Pathophysiology of this association remains unclear. Thymomas are characterized by an extreme variability in histological appearance, as well as in clinical outcomes. The mainstay of therapy is complete surgical resection, which is an important predictor for long-term survival.[1] In our study, thymoma resection was performed in 86.8% of the cases, and 93.9% of these patients they were free from recurrence. The only recurrence of thymoma after surgery happened before AA and immunosuppressive therapy (IST). Overall, IST seems to have little effect on thymoma progression/or relapse especially after thymectomy. Herein, only two reported cases had thymoma progression under IST but these patients had an inoperable thymoma.

AA could occur soon after or late after thymoma resection or could be the first presentation of thymoma. In our study, 20% of the patients had a previous history of PRCA within 3.5 months before AA diagnosis. PRCA could be the first presentation of progressive T-AA but we cannot exclude misdiagnosis. GS is found in 5% of thymoma patients.[2] Herein, 31.6% of evaluable patients had a hypogammaglobulinemia and three had GS, suggesting an association with T-AA: AA should be screened for thymoma in these cases.

The course of AA is often complicated by the development of clonal disorders such as PNH. In our study, minor PNH granulocyte clones were found in 16.7% of investigated patients.

In all the cases reported here, AA was refractory to thymoma surgical resection. In PRCA surgery is performed with the expectation of an improvement of anemia but does not reliably lead to remission.[10] In T-AA, thymectomy alone was also insufficient for normalization of hematopoiesis. In our study, nine patients had first-line treatment with ATG plus CsA (including one associated eltrombopag) and six patients (66.7%) responded. Patients with T-AA had a similar response to first line IST, especially ATG plus CsA, than other types of acquired severe AA.[11] Eltrombopag in first line in association with CsA (n=2), CsA plus ATG (n=1) or alone induced response in two patients (1 CR and 1 PR) as was previously reported in a subset of patients with severe AA.[12,13] The association of romiplostim and danatrol was not efficient in two cases previously refractory to CsA plus ATG as previously published by the FRC for AA.[14] In our study the ORR was higher with CsA use. HSCT allowed CR in all the cases (n=5), which suggest that in case of a patient being eligible for HSCT, thymoma should not be considered as a contraindication, with a possible graft *versus* tumor effect.[15]

Our study has limitations. First, its retrospective design and case identification strategy may bring some bias i) selection of the most complicate cases by using the data of the FRC for AA, ii) data omission. Second, cases from the literature could be composed of successful cases of treatment of T-AA and therefore introduced bias. Third, the retrospective design may limit the interpretation of tolerance data.

In conclusion, thymoma and AA seem rarely associated and it is reasonable to exclude thymoma at AA diagnosis especially in patients older than 50 years. While surgical resection of thymoma is still recommended, it does not reliably lead to hematological remission. Current guidelines for the treatment of idiopathic AA should be followed in T-AA, namely sibling HSCT if a matched family donor is available, and IST in other cases.

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