Anti-FVIII IgG1 Titers Correlate with Bethesda Levels in Acquired Hemophilia A

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Introduction

Acquired hemophilia (AH) is a rare, life-threatening, bleeding disorder caused by autoantibodies that neutralize FVIII procoagulant activity (Inhibitors). The inhibitor level is usually determined using the Bethesda assay however since antibodies from acquired hemophiliacs frequently exhibit type II kinetics their level might be underestimated. Further, the Bethesda assay does not measure non-neutralizing antibodies which also might be present and may accelerate the clearance of FVIII. Because of the low incidence of AH (1:1x10^6) the nature of the total antibody response (neutralizing and non-neutralizing antibodies) is poorly understood. Antibodies to FVIII are polyclonal and previous studies with both congenital and acquired hemophilia have shown that the IgG4 subclass predominates which indicates an immune response that is Th2-driven. In addition, some studies have shown a correlation with the Bethesda titer and the IgG4 subclass but one other did not, presumably because of the presence of non-neutralizing antibodies.

Aims

Our aim was to determine the IgG subclasses of FVIII-specific antibodies in AH and correlate the IgG subclass with the Bethesda levels.

Methods

After IRB approval and informed consent, blood samples from AH patients were collected at their diagnosis. Inhibitor levels were determined by Bethesda assay and FVIII IgG subclasses (IgG1, IgG2, IgG4) were determined by ELISA. Briefly, plates coated with FVIII (1 mg/ml) were incubated overnight, washed then blocked with 5% skim milk in PBS/0.05% Tween-20 (IgG1 or IgG4) or 5% BSA in PBS/0.05% Tween-20 (IgG2). After washing, patient samples were serially diluted in 1% skim milk in PBS/0.05% Tween-20 (IgG1 or IgG4) or 1% BSA in PBS/0.05% Tween-20 (IgG2), applied to the plates and incubated. Plates were washed, then HRP labeled anti human IgG1 (cloneHP6069), IgG4 (clone HP6025) or AP labeled IgG2 (clone HP6002) were added and incubated for 1 hour at 37°C. HRP was detected by addition of TMB substrate and AP was detected by the addition of para-nitrophenylphosphate substrate. The optical densities were determined using a MRX Revelation microplate reader. The antibody titer of any sample was defined as the highest dilution with an OD reading of ≥0.2 (IgG1 or IgG4) and ≥ 0.15 (IgG2). These values were determined by assessing the mean +3 SD of 1:16 dilution of 10 healthy controls (male and female). Correlation coefficients were calculated using Spearman's rank correlation using GraphPad Prism 4 software.

Results

Table 1. Patient Characteristics

Of the 22 patients with acquired hemophilia, 50% were female. The average age was 63.1 and the ages ranged from 37-88. The underlying disorder was idiopathic in 9/22, malignancy in 3/22, autoimmune in 9/22 and HIV in 1/22. There were no pregnancy-associated acquired inhibitor patients. One patient achieved a spontaneous remission, and 11 other patients demonstrated a complete response to treatment. There were 5 deaths, 3 of which were cancer-related.

Patient Characteristics		n	%
Patients (n)		22	
Gender			
	Male	11	50
	Female	11	50
Age (mean, (range))		63.1 (37-88)	
Race/Ethnicity			
	Caucasian (non Hispanic)	12	54.5
	Hispanic	2	9.1
	African American	8	36.4
Underlying Disorder			
	Idiopathic	9	40.9
	Malignancy	3	13.6
	Autoimmune	9	40.9
	HIV	1	4.5
Outcomes			
	Complete response	11	50
	No response	5	22.7
	Spontaneous remission	1	4.5
	Death	5	22.7

Figure 1. Anti FVIII IgG Subclasses

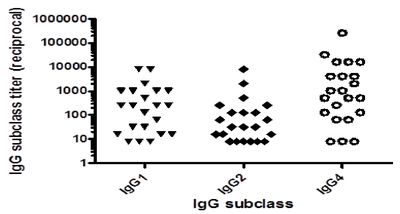

Figure 1. **Titers of Anti-FVIII Antibodies for IgG Subclasses in Acquired Hemophilia**. The IgG subclass titers for twenty-two patients with acquired hemophilia were determined. 19/22 patients had IgG1 and IgG4 titers ≥ 1:16 and 16/22 patients had IgG2 titers ≥ 1:16. For IgG1 and IgG2 the titers ranged from 1:16-1:8192 and for IgG4 they ranged from 1:16-1:262,144.

Figure 2. Correlation between Titers of Anti-FVII IgG Subclasses and Bethesda Units

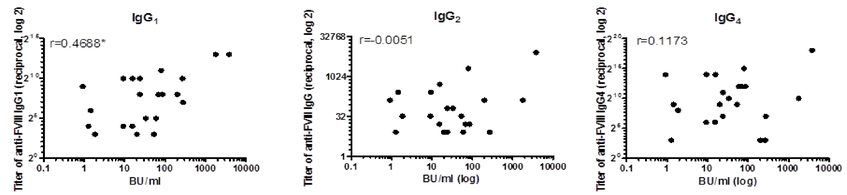

Figure 1. Correlations between Anti FVIII IgG Subclasses and Bethesda Units The only significant correlation between IgG subclass and Bethesda units was found with IgG1 (0.4688). The correlations between IgG2 and IgG4 were 0.0051 and 0.1173, respectively. The asterisk denotes a significance of P<0.05

Conclusions

Greater than 72% of this cohort of acquired hemophiliacs have evidence of IgG1, IgG2 and IgG4 antibodies to FVIII and 86% of these patients have both IgG1 and IgG4 antibody subclasses. We found that the IgG1 subclass titer correlated significantly with the Bethesda levels. However, unlike some previous reports we did not find a significant correlation between the IgG2 and IgG4 subclasses and the Bethesda level. This may be due to the presence of non-neutralizing antibodies.