

Increasing Incidence and Prevalence of Acquired Hemolytic Anemias in Denmark, 1980–2016

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Background: Acquired hemolytic disorders—autoimmune hemolytic anemia (AIHA), cold agglutinin disease (CAD), paroxysmal nocturnal hemoglobinuria (PNH), drug-induced hemolysis (DIHA), and acquired hemolysis not otherwise specified (AHNOS)—are considered rare. Despite their potentially major health implications, data regarding their incidence and prevalence are scarce.

Methods: To fill this gap we collected data regarding all patients with acquired hemolytic disorder diagnoses in 1977–2016 from the Danish National Patient Register. These data were linked with vital and migration status information from the Danish Civil Registration System. From these data combined with annual demographic data for the background population, we calculated age- and sex-specific incidence rates and prevalence proportions of acquired hemolytic disorders for specified time periods.

Results: Our analysis included 5868 patients with acquired hemolytic disorders (2715 with AIHA, 112 CAD, 397 DIHA, 116 PNH, and 2154 AHNOS). The incidence rates per 100 000 person-years in 1980–1993 and 2008–2016 were 0.81 and 1.77 for AIHA, 0.31 and 0.12 for DIHA, and 0.04 and 0.08 for PNH, respectively. The 2008–2016 CAD incidence rate was 0.18/100 000 person-years, CAD diagnosis code was not defined before 1994. All incidence rates increased with age. The prevalence proportion per 100 000 persons in 1980 and 2015 was 2.52 and 17.01 for AIHA, 0.80 and 1.50 for DIHA, and 0.18 and 1.04 for PNH. CAD prevalence in 2015 was 1.04/100 000 persons.

Conclusion: Acquired hemolytic anemia incidence rates and prevalence proportions with the exception of DIHA are markedly increasing.

Keywords: autoimmune hemolytic anemia, cold agglutinin disease, paroxysmal nocturnal hemoglobinuria, drug-induced hemolytic anemia, incidence, prevalence

Introduction

Acquired hemolytic disorders—comprising autoimmune hemolytic anemia (AIHA), cold agglutinin disease (CAD), paroxysmal nocturnal hemoglobinuria (PNH), drug-induced hemolysis, and acquired hemolysis not otherwise specified (NOS)—are considered rare and are reportedly associated with increased mortality.^{1–10} However, few data are available regarding the incidence and prevalence of these disorders.

Two studies published in 1973 and 2010 describe an approximate AIHA incidence rate of 0.5–1 per 100 000 person-years^{1,2} but contemporary nationwide estimates are lacking. AIHA prevalence is also sparsely described.³ Similarly, CAD incidence is reported in only one study, as 0.1/100 000 person-years during the years 1995–2004.⁴ PNH incidence was estimated to be 0.13/100 000 person-years during 1991–2006.⁵

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With regard to drug-induced hemolytic anemia, one study suggests a declining incidence of this adverse drug effect, possibly mirroring the declining use of high-dose penicillin and methyldopa; however, a more recent study described an increased incidence, possibly linked to the introduction of treatment with cephalosporins.⁶

In the present study, we describe the acquired hemolytic anemia incidence and prevalence trajectories within a nationwide cohort from 1980 to 2016.

Methods

For this register-based study, we linked routinely collected individual-level administrative health data from the Danish National Patient Register (Patient Register) with information regarding death, migration, and demographics from the Danish Civil Registration System.^{11,12} Denmark provides universal tax-funded healthcare for all inhabitants. All hospitals, public or private, report to the Patient Register and other administrative registers.¹³ The few private hospitals in Denmark do not manage blood disorders.¹² A detailed description of the registers is available in the [Online Supplementary](#). We used the checklist “reporting of studies conducted using observational routinely-collected health data” (RECORD) statement to structure and report our findings.¹⁴

Inclusion

The Patient Register has recorded all diagnoses from hospitalizations since 1977, and from out-patient contacts since 1994. Diagnoses are classified according to the International Classification of Diseases (ICD) 8th revision until 1994, and 10th revision thereafter.¹² Inclusion of patient from the Patient Register was based on previously validated diagnosis codes for specific acquired hemolytic disorders.¹⁵ Establishing an accurate diagnosis of hemolytic subtype may require several hospital visits, and result in registrations of multiple diagnostic codes. Based on the validation study, patients having more than one diagnosis code compatible with acquired hemolytic anemia were assigned the diagnosis with the highest positive predictive value (PPV) from departments of hematology, pediatrics, and internal medicine (further details in [Online Supplementary](#)).¹⁵ The first date of final diagnosis was used as the inclusion date in this study ([Supplementary Figure 1](#)).

We retrieved information about these potential underlying causes as part of the comorbidity information from the Patient Register. To define a state of secondary acquired hemolysis, diseases had to be recorded any time before and

up to 100 days after inclusion date. [Supplementary Table 2](#) lists the diagnoses defining secondary hemolysis.

Exclusion

Patients were excluded if they were registered only with congenital hemolytic disorders. We have previously reported incidence and prevalence of combined AIHA and immune thrombocytopenia (Evans syndrome), based on 263 patients from the same data sources.^{16,17} These patients are excluded from the present analysis.

Outcomes

The primary outcomes were incidence rates and prevalence proportions. Incidence rates were calculated for the time-periods 1980–1993, 1994–2007, and 2008–2016. Prevalence proportions were calculated on the 1st of January in 1980, 2000, and 2015. The secondary outcome was median survival after diagnosis of acquired hemolysis.

Statistics

Data were managed and analyzed using Stata 15.1.¹⁸ Incidence rates were calculated from the cumulative incidences during the time-periods 1980–1993, 1994–2007, and 2008–2016. Prevalence proportions were calculated as the number of patients alive with an acquired hemolysis diagnosis on the 1st of January in 1980, 2000, and 2015. Both incidence rate and prevalence proportion were reported per 100 000 persons using stratified census data as denominator. The incidence rate and prevalence proportions for each diagnosis were stratified by sex and age at the time of diagnosis (<20 years, 20–50 years, and >50 years old). We evaluated changes in overall incidence rates and prevalence proportions using negative binomial regression, estimating incidence rate ratios (IRR) and prevalence proportion ratios (PPR).¹⁹ However, if the dispersion parameter was indistinguishable from zero, the regressions were simplified to Poisson regressions.²⁰ Median survival time from the date of hemolysis diagnosis was estimated using the Kaplan–Meier method. Further details and sensitivity tests are presented in the [Online Supplementary](#).

Approval and Ethics

In Denmark, research based on registry data without direct patient interaction does not require scientific ethical approval. This study was approved by the Danish Data Protection Agency (reference: 17/10885). Danish law prohibits making national health data publicly available.

Results

The population of Denmark increased from 5122 005 persons (50.6% women) in 1980 to 5707 251 (50.2% women) in 2016.²¹ From the Patient Register, we retrieved 30,520 patients with hemolysis or immune thrombocytopenia ([Supplementary Table 1](#) and [Supplementary Figure 1](#)). Applying our study criteria, we included 5868 patients with an acquired hemolysis diagnosis ([Table 1](#) and [Supplementary Table 3](#)). [Table 1](#) presents basic characteristics, demographic information, and median survival. Mean follow-up time was 7.4 years, with a total follow-up time of 43,696 years.

Using the main diagnostic model, 2715 patients were registered with AIHA, 112 with CAD, 116 with PNH, and 397 with drug-induced hemolysis. Among the remaining 2528 patients, 2154 were identified as having acquired hemolysis NOS, and 374 had very rare otherwise defined hemolytic anemias (eg paroxysmal cold hemolysis, march hemoglobinuria, etc.). The distribution of hemolytic subtypes is summarized in [Table 1](#), [Supplementary Table 3](#), and [Supplementary Figure 1](#). Underlying diseases potentially associated with hemolytic anemia was recorded in 40.8% [95% CI: 39.0; 42.7] of patients with AIHA. The proportion of patients diagnosed on a department of hematology increased for all diseases during the study time, but was most pronounced for the specific diagnosis of AIHA, CAD, PNH, data not shown.

Incidence

[Table 2](#) presents the incidence rates of specified hemolysis diagnoses. Using the time-period 1980–1993 as reference, nearly all types of acquired hemolysis showed an increasing IRR during the study period ([Table 2](#), [Supplementary](#)

[Table 4](#), and [Supplementary Figure 2](#)). As an exception, the incidence rate of drug-induced hemolysis exhibited an overall decrease of 73.4% from 1980–1993 to 1994–2007, corresponding to IRRs of 0.27 [95% CI: 0.20; 0.35] for 1994–2007 and 0.37 [95% CI: 0.29; 0.47] for 2008–2016 ([Supplementary Table 6](#)). The incidence rates of all hemolytic diseases increased with age ([Table 2](#)). AIHA incidence was significantly higher among women than men, whereas other hemolysis subtypes showed equal incidence rates in men and women ([Table 2](#)).

Prevalence

[Table 3](#) presents the prevalence proportions for specific acquired hemolysis diagnoses, and [Figure 1](#) shows the annual overall prevalence proportions from 1980 to 2016 for each diagnosis. Prevalence proportions increased during the study period for all hemolytic subtypes, except for drug-induced hemolysis ([Table 3](#), [Figure 1](#), [Supplementary Tables 5](#) and [7](#), and [Supplementary Figure 3](#)). Showing no evidence of overdispersion, Poisson regression confirmed the increases in the estimated annual prevalence proportion. All diagnoses, except drug-induced hemolysis, showed a continuous increase ([Supplementary Table 5](#) and [Supplementary Figure 2](#)). AIHA, CAD, and drug-induced hemolysis were most prevalent among patients >50 years of age, whereas PNH was most prevalent among patients 20–50 years of age ([Table 3](#)).

Sensitivity Analyses

The results of sensitivity analyses are presented in the [Online Supplement](#).

Table 1 Characteristics of Patients with Acquired Hemolytic Disorders in Denmark, 1980–2016

	AIHA, n = 2715	CAD, n = 112	Drug Induced, n = 397	PNH, n = 116	Acquired Hemolysis NOS, n = 2154	Other Defined Hemolysis, n = 374
% (95% CI)						
Women	55.9 (54.0; 57.8)	51.8 (42.1; 61.3)	62.0 (57.0; 66.8)	50.0 (40.6; 59.4)	53.6 (51.5; 55.7)	46.0 (40.9; 51.2)
Deceased	63.8 (61.9; 65.6)	39.6 (30.5; 49.4)	79.6 (75.3; 83.5)	41.6 (32.4; 51.2)	66.9 (64.9; 68.9)	46.2 (41.0; 51.4)
Median (IQR)						
Age at diagnosis, years	68.7 (53.8; 78.3)	71.9 (61.0; 78.6)	66.6 (53.1; 76.9)	48.4 (31.7; 67.0)	67.4 (48.5; 78.3)	57.1 (26.4; 73.1)
Age at death, years	78.6 (69.8; 85.1)	80.8 (74.5; 87.7)	77.2 (65.4; 84.4)	71.5 (56.5; 79.6)	77.3 (68.2; 84.9)	76.3 (64.3; 84.5)
Median survival, years	6.3 (1.3; 21.9)	8.8 (3.6; na) ^a	4.5 (0.9; 16.2)	23.2 (6.8; na) ^a	4.9 (0.9; 21.7)	13.5 (2.0; na) ^a

Notes: ^aUpper bound in confidence intervals for median survival could not be calculated for CAD, drug-induced hemolysis, PNH, and the group of other defined hemolysis. **Abbreviations:** AIHA, autoimmune hemolytic anemia; CAD, cold agglutinin disease; CI, confidence interval; na, not applicable; NOS, not otherwise specified; PNH, paroxysmal nocturnal hemoglobinuria.

Table 2 Incidence of Acquired Hemolytic Diseases in Denmark, 1980–2016

		Incidence per 100 000 Person-Years (95% CI)		
		1980–1993	1994–2007	2008–2016
AIHA				
	All	0.81 (0.74; 0.87)	1.43 (1.35; 1.52)	1.77 (1.66; 1.89)
	Age <20	0.25 (0.19; 0.32)	0.26 (0.19; 0.33)	0.35 (0.27; 0.46)
	Age 20–50	0.25 (0.20; 0.30)	0.43 (0.36; 0.50)	0.51 (0.42; 0.61)
	Age >50	1.76 (1.60; 1.93)	3.12 (2.91; 3.33)	3.34 (3.10; 3.59)
	Female	0.86 (0.77; 0.96)	1.50 (1.38; 1.62)	1.71 (1.56; 1.87)
	Male	0.64 (0.56; 0.73)	1.18 (1.07; 1.29)	1.48 (1.34; 1.63)
CAD				
	All	na	0.03 (0.02; 0.04)	0.18 (0.15; 0.22)
	Age <20	na	0.00 (0.00; 0.02)	0.00 (0.00; 0.02)
	Age 20–50	na	0.01 (0.00; 0.02)	0.03 (0.01; 0.06)
	Age >50	na	0.07 (0.04; 0.10)	0.39 (0.31; 0.49)
	Female	na	0.03 (0.02; 0.06)	0.16 (0.12; 0.21)
	Male	na	0.02 (0.01; 0.04)	0.17 (0.12; 0.22)
Drug Induced				
	All	0.31 (0.27; 0.36)	0.08 (0.06; 0.11)	0.12 (0.09; 0.15)
	Age <20	0.02 (0.00; 0.05)	0.04 (0.02; 0.08)	0.02 (0.00; 0.05)
	Age 20–50	0.10 (0.07; 0.14)	0.03 (0.02; 0.06)	0.05 (0.02; 0.08)
	Age >50	0.74 (0.64; 0.86)	0.15 (0.11; 0.20)	0.21 (0.15; 0.28)
	Female	0.35 (0.29; 0.41)	0.09 (0.06; 0.12)	0.11 (0.07; 0.15)
	Male	0.23 (0.19; 0.29)	0.07 (0.05; 0.10)	0.10 (0.07; 0.15)
PNH				
	All	0.04 (0.03; 0.06)	0.05 (0.03; 0.06)	0.08 (0.06; 0.11)
	Age <20	0.01 (0.00; 0.04)	0.01 (0.00; 0.03)	0.01 (0.00; 0.03)
	Age 20–50	0.04 (0.02; 0.06)	0.04 (0.03; 0.07)	0.08 (0.05; 0.12)
	Age >50	0.06 (0.03; 0.10)	0.06 (0.03; 0.09)	0.09 (0.06; 0.14)
	Female	0.04 (0.02; 0.07)	0.04 (0.02; 0.06)	0.07 (0.04; 0.11)
	Male	0.04 (0.02; 0.07)	0.05 (0.03; 0.07)	0.07 (0.04; 0.11)
Acquired Hemolysis NOS				
	All	0.81 (0.74; 0.87)	0.99 (0.92; 1.07)	1.28 (1.18; 1.38)
	Age <20	0.34 (0.27; 0.43)	0.30 (0.23; 0.38)	0.34 (0.26; 0.44)
	Age 20–50	0.25 (0.20; 0.31)	0.34 (0.28; 0.41)	0.46 (0.38; 0.55)

(Continued)

Table 2 (Continued).

		Incidence per 100 000 Person-Years (95% CI)		
		1980–1993	1994–2007	2008–2016
	Age >50	1.67 (1.51; 1.84)	2.01 (1.85; 2.19)	2.24 (2.05; 2.45)
	Female	0.81 (0.72; 0.90)	1.01 (0.91; 1.11)	1.16 (1.04; 1.29)
	Male	0.69 (0.61; 0.78)	0.85 (0.76; 0.94)	1.15 (1.03; 1.28)
Other Defined Hemolysis				
	All	0.02 (0.01; 0.03)	0.26 (0.22; 0.29)	0.33 (0.28; 0.38)
	Age <20	0.02 (0.01; 0.05)	0.21 (0.15; 0.28)	0.17 (0.11; 0.24)
	Age 20–50	0.02 (0.01; 0.04)	0.12 (0.08; 0.16)	0.14 (0.10; 0.20)
	Age >50	0.01 (0.00; 0.04)	0.38 (0.31; 0.46)	0.49 (0.40; 0.59)
	Female	0.02 (0.01; 0.03)	0.23 (0.18; 0.28)	0.26 (0.21; 0.33)
	Male	0.02 (0.01; 0.04)	0.25 (0.21; 0.31)	0.32 (0.26; 0.40)

Notes: Incidences of acquired hemolytic diseases estimated as cumulative new diagnosed patients in each period, using cumulative stratified census data as the denominator. CAD diagnosis was not defined in the ICD before 1994.

Abbreviations: AIHA, autoimmune hemolytic anemia; CAD, cold agglutinin disease; CI, confidence interval; na, not applicable; NOS, not otherwise specified; PNH, paroxysmal nocturnal hemoglobinuria.

Discussion

Our present study was the first to examine basic measures of the frequency of acquired hemolytic anemia within a nationwide population. With regards to the subtypes of hemolytic anemia AIHA, CAD, PNH, and acquired hemolysis NOS, and the residual group of other defined hemolysis, we found that the incidence rates and prevalence proportions markedly increased during the 36-year study period. Below we discuss the findings for each of the hemolytic subtypes.

AIHA

AIHA incidence rates per 100 000 person-years increased from 0.8 during the period 1982–1993, to 1.4 in 1994–2007, and 1.8 in 2008–2016. These results are comparable with two previous studies reporting AIHA incidences ranging from 0.5 to 1 per 100 000 person-years in Sweden during 1964–1968¹ and California during 1998–2004.² However, our present results are the first finding of a continuous increase of incidence during our study period.

The increasing incidence rate may be related to a more comprehensive diagnostic work-up, increased awareness, and a true increase of disease incidence and prevalence. The increase of AIHA incidence was clearly greater than the increase of acquired hemolysis NOS incidence during the same time period (Figure 1). This may suggest that the

AIHA increase was partly due to a more comprehensive diagnostic work-up, in that patients exhibiting hemolysis during the latter part of the study period underwent a diagnostic work-up including DAT-test and other potential causes were excluded. Thus, they were more frequently diagnosed specifically with AIHA, compared with the earlier study period. Supporting this notion, we previously found that the incidence of Evans syndrome increased at the same pace during the same time-period.¹⁶ However, it is also conceivable that the population incidence of AIHA increased, similar to the increased incidence of other autoimmune diseases described during the same period.^{22–26} We cannot from our existing data estimate the incidence and prevalence of all predisposing disease to assess the potential impact on AIHA; however, the results of a post hoc analysis (Supplementary Figures 4 and 5) indicated that the incidence and prevalence of secondary AIHA increased disproportionally more than corresponding measures of occurrence of primary AIHA. In 2016 secondary AIHA accounted approximately for one-third of all prevalent AIHA diagnoses, and had an incidence rate equal to that of primary AIHA. This increase could be related to a more comprehensive clinical care and work-up of patients with predisposing conditions whereby AIHA is more often diagnosed among these patients. This could further be amplified by an increase in prevalence of patients with

Table 3 Prevalence of Acquired Hemolytic Diseases in Denmark, 1980–2015

		Prevalence per 100 000 (95% CI)		
		1980	2000	2015
AIHA				
	All	2.52 (2.10; 2.99)	9.46 (8.65; 10.32)	17.01 (15.96; 18.12)
	Age <20	1.77 (1.15; 2.59)	7.92 (6.44; 9.63)	12.39 (10.56; 14.44)
	Age 20–50	0.88 (0.53; 1.38)	5.39 (4.50; 6.42)	11.27 (9.93; 12.73)
	Age >50	5.59 (4.46; 6.92)	16.17 (14.32; 18.19)	26.38 (24.21; 28.70)
	Female	2.78 (2.17; 3.50)	10.57 (9.38; 11.87)	19.06 (17.49; 20.73)
	Male	2.25 (1.71; 2.92)	8.31 (7.25; 9.49)	14.94 (13.55; 16.44)
CAD				
	All	na	0.02 (0.00; 0.10)	1.04 (0.79; 1.34)
	Age <20	na	0.00 (0.00; 0.29)	0.00 (0.00; 0.28)
	Age 20–50	na	0.04 (0.00; 0.24)	0.39 (0.18; 0.75)
	Age >50	na	0.00 (0.00; 0.22)	2.43 (1.81; 3.21)
	Female	na	0.04 (0.00; 0.21)	1.12 (0.77; 1.59)
	Male	na	0.00 (0.00; 0.14)	0.96 (0.63; 1.40)
Drug Induced				
	All	0.80 (0.57; 1.09)	1.48 (1.17; 1.85)	1.50 (1.20; 1.86)
	Age <20	0.07 (0.00; 0.38)	0.71 (0.33; 1.35)	1.06 (0.58; 1.79)
	Age 20–50	0.37 (0.16; 0.73)	0.85 (0.52; 1.31)	1.48 (1.03; 2.07)
	Age >50	2.13 (1.46; 3.01)	2.92 (2.17; 3.85)	1.80 (1.27; 2.48)
	Female	1.31 (0.91; 1.83)	2.08 (1.57; 2.70)	1.97 (1.48; 2.55)
	Male	0.28 (0.11; 0.57)	0.87 (0.55; 1.31)	1.03 (0.69; 1.48)
PNH				
	All	0.18 (0.08; 0.33)	0.69 (0.49; 0.96)	1.04 (0.79; 1.34)
	Age <20	0.00 (0.00; 0.25)	0.16 (0.02; 0.57)	0.38 (0.12; 0.89)
	Age 20–50	0.23 (0.08; 0.54)	0.93 (0.59; 1.41)	1.70 (1.21; 2.33)
	Age >50	0.27 (0.07; 0.68)	0.76 (0.40; 1.30)	0.73 (0.41; 1.20)
	Female	0.15 (0.04; 0.39)	0.70 (0.42; 1.10)	1.09 (0.74; 1.54)
	Male	0.20 (0.06; 0.46)	0.68 (0.40; 1.08)	1.00 (0.66; 1.44)
Acquired Hemolysis NOS				
	All	2.52 (2.10; 2.99)	7.26 (6.56; 8.02)	12.51 (11.60; 13.47)
	Age <20	1.15 (0.67; 1.85)	9.11 (7.52; 10.93)	14.21 (12.25; 16.40)
	Age 20–50	1.44 (0.98; 2.05)	4.88 (4.03; 5.86)	10.09 (8.83; 11.48)

(Continued)

Table 3 (Continued).

		Prevalence per 100 000 (95% CI)		
		1980	2000	2015
	Age >50	5.39 (4.28; 6.70)	9.17 (7.79; 10.72)	14.12 (12.54; 15.84)
	Female	2.74 (2.14; 3.45)	7.75 (6.74; 8.88)	13.13 (11.83; 14.53)
	Male	2.29 (1.74; 2.96)	6.76 (5.80; 7.83)	11.88 (10.64; 13.23)
Other Defined Hemolysis				
	All	0.08 (0.02; 0.20)	1.48 (1.17; 1.85)	3.30 (2.85; 3.81)
	Age <20	0.07 (0.00; 0.38)	2.14 (1.41; 3.11)	5.32 (4.15; 6.72)
	Age 20–50	0.09 (0.01; 0.34)	1.15 (0.76; 1.67)	2.45 (1.85; 3.18)
	Age >50	0.07 (0.00; 0.37)	1.46 (0.94; 2.15)	2.97 (2.27; 3.81)
	Female	0.04 (0.00; 0.21)	1.11 (0.75; 1.59)	2.91 (2.32; 3.61)
	Male	0.12 (0.02; 0.35)	1.86 (1.38; 2.46)	3.70 (3.02; 4.48)

Notes: Prevalence proportions were estimated as the number of living persons assigned the diagnosis at the latest on the 1st of January in each of the years 1980, 2000, and 2015, with stratification by age and sex, with population denominators derived from census data. CAD diagnosis was not defined in the ICD before 1994.

Abbreviations: AIHA, autoimmune hemolytic anemia; CAD, cold agglutinin disease; CI, confidence interval; na, not applicable; NOS, not otherwise specified; PNH, paroxysmal nocturnal hemoglobinuria.

predisposing conditions such as autoimmune diseases or malignancies.^{24–28}

In a previous study, AIHA prevalence was estimated to be 17/100 000 persons in Denmark in 2001.³ In our present analysis, we estimated that the AIHA prevalence was 9.5/100 000 persons in 2000. This estimate was lower due to our exclusion of patients with diagnoses made exclusively at non-medical departments.¹⁵ Importantly, our present approach was based on the results of a previous validation study performed in the same setting and with the same registry sources as in the present study.¹⁵ More extensive inclusion, based on less strict criteria, would likely overestimate the AIHA incidence and prevalence.¹⁵

CAD

The increased CAD incidence rate in our study, from 0.03 to 0.18/100 000 person-years, may partly reflect that the specific CAD diagnosis code was first applied in 1994. Our results may reflect a delay in routine application of this coding. To our knowledge, CAD incidence has only been assessed in two previous studies. The first study is from Norway, covering 1995–2004. The reported incidence was 0.1/100 000 person-years, which is in line with our current results from the latest time-period.⁴ Further, a previous Danish study using the Patient Register in the years 1999–2013 reported results very

similar to ours,²⁹ even though that study, unlike the present study, included diagnoses from non-medical departments.

The Norwegian study also revealed a CAD prevalence of 1.6/100 000 persons in 2005, comparable to our present result of 1.04 per 100 000 in 2015.⁴ This minor difference in CAD prevalence may be explained by the shorter median survival of these patients in Denmark (8.8 years) compared with Norway (12.5 years) (Table 1), which is probably attributable to the higher age at diagnosis in our cohort compared to previous reports.^{4,30}

PNH

One previous study reported the PNH incidence rate to be 0.13/100 000 person-years in 1991–2006 in Yorkshire, United Kingdom,⁵ which is comparable with our results of 0.05–0.08/100 000 person-years in 1994–2016.⁵ The same study from the UK found a PNH prevalence proportion of 1.59/100 000 persons, which is higher than the equivalent estimates in our study (0.69–1.04/100 000 persons in 2000 and 2015).⁵ These estimates may differ due to different diagnostic approaches in the two countries and/or a true difference in factors that determine prevalence (ie disease incidence and survival).

Notably, both the incidence and prevalence of PNH will be higher if patients are registered with a PNH diagnosis due to very small PNH clones with unclear clinical

Prevalence of

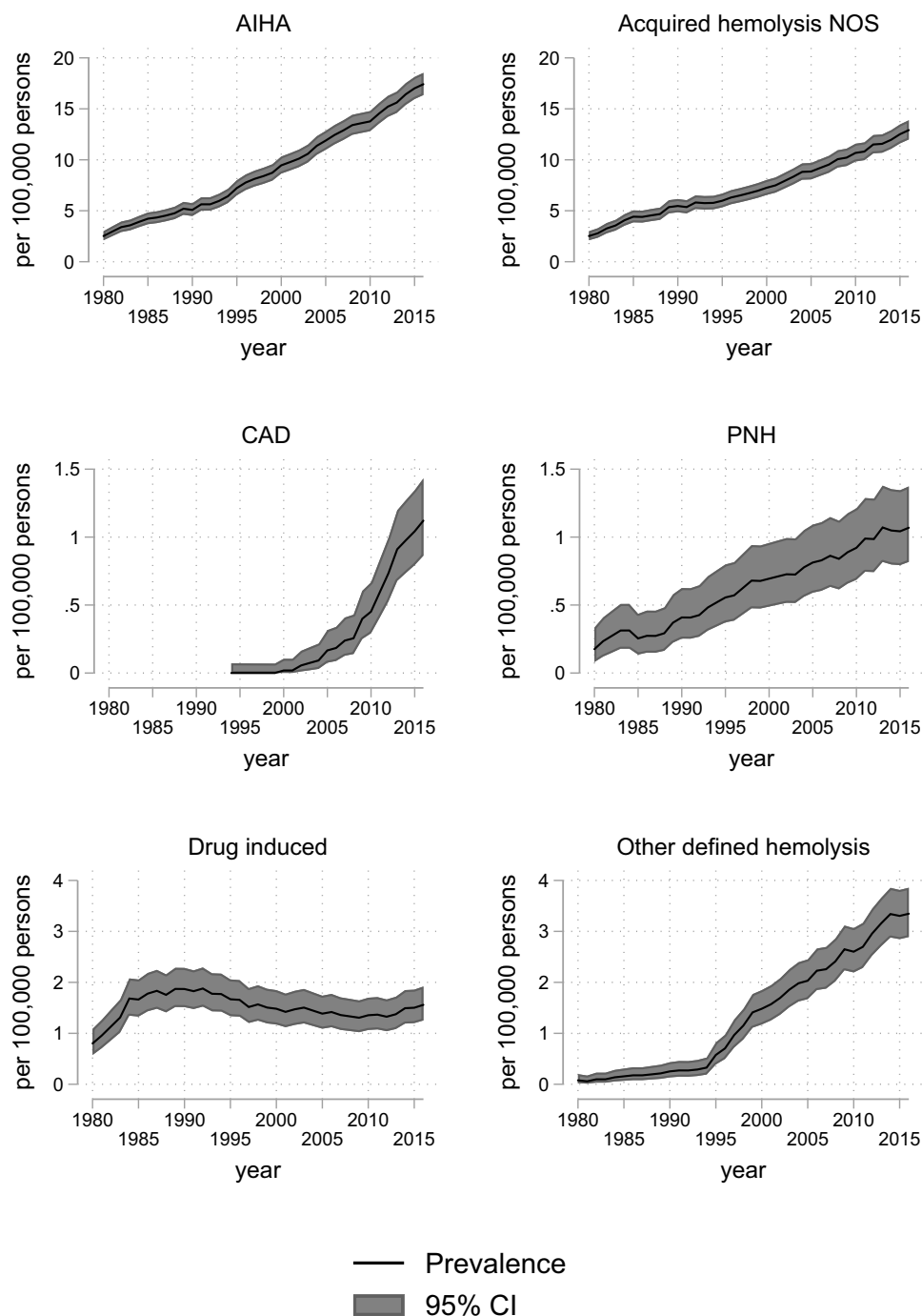


Figure 1 Prevalence of acquired hemolysis in Denmark, 1980–2016.

Notes: The overall prevalence proportion with 95% confidence intervals for all acquired hemolytic diseases, calculated on 1st of January each year, using census data as the denominator. The 95% confidence intervals were calculated using the Clopper–Pearson method. CAD diagnosis was not defined in the ICD before 1994. Data were based on a national cohort of patients from Denmark diagnosed in 1980–2016.

Abbreviations: AIHA, autoimmune hemolytic anemia; CAD, cold agglutinin disease; CI, confidence interval; NOS, not otherwise specified; PNH, paroxysmal nocturnal hemoglobinuria.

significance eg in conjunction with aplastic anemia (AA) or myelodysplastic syndrome (MDS).^{5,31–34} This overlap in the diagnosis code for PNH between “classic PNH” and

“PNH associated with other bone marrow disease” is depicted in [Supplementary Figures 6](#) and [8](#), where the incidence of PNH associated with AA or MDS increases

markedly in the latest period. The increase in PNH in this selected group could indicate an increased use of sensitive flow cytometry with increased finding of smaller PNH clones.³⁴ Further, the prevalence of PNH associated with AA or MDS begins to rise after 2005 ([Supplementary Figures 7 and 9](#)), and in 2015 PNH associated with AA or MDS accounts for roughly 10–20% of all prevalent PNH patients. Moreover, the incidence of PNH without AA or MDS in [Supplementary Figures 6 and 8](#), increases steadily during the study period, which could indicate both increasing incidence and increased awareness. The latter could be related to new PNH treatment options (for example eculizumab³⁵ which has been available since 2007). The new treatment options could partly explain the increasing prevalence due to improved survival and—to some degree—increased PNH diagnoses, as physicians may consider referring and correct coding to be more important if the disease is considered treatment-modifiable. Finally, since PNH is truly rare, even small random variations in the number of patients with the disorder may considerably impact measures of PNH occurrence.

Drug-Induced Hemolytic Anemia

Due to the sparse data, we combined the diagnoses of immune and non-immune drug-induced hemolytic anemia into one entity. Our results corroborated the previously proposed trend in drug-induced hemolysis over time.⁶ The decreased incidence rate supports Garratty's hypothesis that the abolishment of high-dose penicillin and methyldopa would lead to a decreased incidence of drug-induced hemolytic anemia.⁶ However, our data lacked granularity in terms of assessing which medications were potentially involved in drug-induced hemolysis. Furthermore, the results regarding the drug-induced hemolysis should be interpreted cautiously, as the PPV of this diagnosis was lower than that of other acquired hemolytic anemias.

Acquired Hemolysis NOS

The majority of patients with acquired hemolysis NOS was throughout our study period diagnosed without referral to hematology departments, unlike the similarly sized group of AIHA patients that over the study period were increasingly referred to specialized hematology departments. As specialized hemolysis diagnosing in Denmark is mostly done by departments of hematology, this lower referral rate could explain the unspecified subtype as the result of a less comprehensive diagnostic work-up. A previous validation study has indicated that acquired

hemolysis is very likely to have been present, if the patient was given a diagnosis of acquired hemolysis.¹⁵ From a probabilistic view point this unspecified acquired hemolysis probably mostly comprises patients with AIHA (including DAT-negative AIHA) that did not complete a sufficient diagnostic work-up, and a group of various less specific acquired hemolytic conditions, eg drug-related, transfusion-related, angio-pathic and mechanical hemolysis.

Sensitivity Analyses

The small non-significant differences in prevalence between the main model and the sensitivity model indicate that the choice of model had limited impact on the estimated proportions of the most common hemolytic diseases. The differences were somewhat greater for rare hemolytic disorders, such as CAD and PNH. This likely reflects that these very rare diseases have a longer diagnostic work-up, such that using the first hemolytic diagnosis or the most reliable diagnosis will have a notable impact.

Strengths and Limitations

Our present analysis relied on the diagnosis with the highest PPV in patients diagnosed with multiple different acquired hemolysis diagnosis (as opposed to the approach in the sensitivity model), which may have introduced a survivorship bias that could have affected the incidence. Specific diagnoses are assigned when sufficient clinical and para-clinical information is available. Patients with severe acquired hemolysis may die before completion of the work-up necessary to reach a specific diagnosis, which could lower the incidence of specific hemolytic subtypes, and lead to overestimation of survival due to non-inclusion of the most severe cases. However, in our sensitivity analyses, use of the first diagnosis date for follow-up yielded results comparable to the results of our main analyses, suggesting that neither of these potential misclassifications majorly impacted the study results.

The transition from ICD-8 to ICD-10 could also introduce a bias; however, since most of the diseases were equally defined in both systems ([Supplementary Table 1](#)), any impact was probably minor. However, the ICD-10 introduced more detailed diagnosis codes, which in itself may have encouraged a more thorough diagnostic work-up. Additionally, changes over time in the degree or content of diagnostic work-up (eg due to new technology) may cause diagnostic drift, and thus exaggerate temporal trends in

incidence rates based solely on data derived from registries.³⁶ We think that it is unlikely that diagnostic drift played a major role in this study, since there were only limited improvements in the diagnostic work-up of the majority of acquired hemolytic anemias during the study period, with PNH being a potential exception as explained in the PNH part of the discussion.^{8,33,37,38} Notably, due to ageism, older patients were potentially more likely to be underserved with regard to extensive work-up in the 1980s compared to in more recent decades.

AIHA and CAD impose a specific challenge regarding definition and diagnosis. Recently attempts have been made to define AIHA and CAD in order to serve both clinical and research purposes.^{15,39,40} In a previous validation study, we assessed active hemolysis based on blood test results of haptoglobin, lactate dehydrogenase, bilirubin and cell-free hemoglobin, a definition comparable to that later recommended by Jäger et al.⁴⁰ The ICD-8 and ICD-10 codes for AIHA are in principle a diagnosis of unspecified autoimmune hemolysis excluding drug and transfusion related hemolysis.^{15,40} The ICD-8 and ICD-10 codes for AIHA correspond in principle to a diagnosis of unspecified autoimmune hemolysis (excluding drug and transfusion-related hemolysis).^{15,40} The Danish adaptation of the ICD-10 contains a specific sub-category for CAD (D591A), but not for other types of AIHA.¹⁵ The introduction of the specific subcategory for CAD in 1994 has made it possible to find patients who have a very high probability of having CAD. However, it is highly probable that a fraction of patients with CAD are less accurately coded as AIHA. As a consequence of this overlap in diagnostic categories the diagnosis of AIHA in the Patient Register is probably a heterogeneous group containing warm, cold and mixed antibody serotypes, and thereby a group of patients less strictly defined than what would be recommended in clinical trials.^{39,40} This overlap in diagnosis categories and codes mentioned above probably also is the explanation for our finding of fewer CAD patients compared to AIHA than previously reported.^{4,40,41}

We only accepted diagnoses issued from departments of hematology, pediatrics, and internal medicine, as these departments make the most valid diagnoses.¹⁵ Due to this choice, our analyses excluded patients not referred to one of the above-mentioned departments but who had received a correct diagnosis from another department (eg surgical ward). This likely led to some degree of underestimation of the incidence and prevalence of acquired hemolytic anemias. However, in our previous validation study, we found that it is very rare for a patient with a correct

diagnosis not to be in contact with one of the included departments.¹⁵ Finally, some acquired hemolytic disorders, such as PNH or CAD, may present with vague symptoms and therefore remain undiagnosed. Overall, we consider our present estimates of the incidence and prevalence of acquired hemolytic anemias to be conservative.

Conclusion

With regards to AIHA, CAD, PNH, and acquired hemolysis NOS, and the residual group of other identifiable hemolytic disorders, we found that the incidence rate and prevalence proportion increased over the study periods, both in general and in all age groups and for both sexes. Notably, during our study period, AIHA more than doubled in incidence and more than tripled in prevalence, while the incidence of drug-induced hemolysis decreased. Our present definition of hemolytic disorders in administrative registries may facilitate future studies of outcome in these rare patients. Moreover, this new knowledge from a large nationwide cohort adds to the limited available information regarding the epidemiology of hemolytic diseases, and provides contemporary data for research and healthcare planning.

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Author Contributions

All authors participated in defining the Danish Hemolysis Cohort. DLH and HF conceived the study. All authors participated in designing the study. DLH performed the analyses, aided by SM. The first draft of the paper was written by DLH, and all authors participated in writing subsequent drafts. All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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References

- Böttiger LE, Westerholm B. Acquired haemolytic anaemia. I. Incidence and aetiology. *Acta Med Scand.* 1973;193(3):223–226. doi:10.1111/j.0954-6820.1973.tb10567.x
- Klein NP, Ray P, Carpenter D, et al. Rates of autoimmune diseases in Kaiser Permanente for use in vaccine adverse event safety studies. *Vaccine.* 2010;28(4):1062–1068. doi:10.1016/j.vaccine.2009.10.115
- Eaton WW, Rose NR, Kalaydjian A, Pedersen MG, Mortensen PB. Epidemiology of autoimmune diseases in Denmark. *J Autoimmun.* 2007;29(1):1–9. doi:10.1016/j.jaut.2007.05.002
- Berentsen S, Ulvestad E, Langholm R, et al. Primary chronic cold agglutinin disease: a population based clinical study of 86 patients. *Haematologica.* 2006;91(4):460–466.
- Hill A, Platts PJ, Smith A, et al. The incidence and prevalence of paroxysmal nocturnal hemoglobinuria (PNH) and survival of patients in Yorkshire. *Blood.* 2006;108(11):985. doi:10.1182/blood.V108.11.985.985
- Garratty G. Immune hemolytic anemia associated with drug therapy. *Blood Rev.* 2010;24(4–5):143–150. doi:10.1016/j.blre.2010.06.004
- Cooper GS, Stroehla BC. The epidemiology of autoimmune diseases. *Autoimmun Rev.* 2003;2(3):119–125. doi:10.1016/S1568-9972(03)00006-5
- Dacie SJ. The immune haemolytic anaemias: a century of exciting progress in understanding. *Br J Haematol.* 2001;114(4):770–785. doi:10.1046/j.1365-2141.2001.02945.x
- Gehrs BC, Friedberg RC. Autoimmune hemolytic anemia. *Am J Hematol.* 2002;69(4):258–271. doi:10.1002/ajh.10062
- Jacobson DL, Gange SJ, Rose NR, Graham NM. Epidemiology and estimated population burden of selected autoimmune diseases in the United States. *Clin Immunol Immunopathol.* 1997;84(3):223–243. doi:10.1006/clin.1997.4412
- Schmidt M, Pedersen L, Sørensen HT. The Danish Civil Registration System as a tool in epidemiology. *Eur J Epidemiol.* 2014;29(8):541–549. doi:10.1007/s10654-014-9930-3
- Schmidt M, Schmidt SA, Sandegaard JL, Ehrenstein V, Pedersen L, Sørensen HT. The Danish National Patient Registry: a review of content, data quality, and research potential. *Clin Epidemiol.* 2015;7:449–490. doi:10.2147/CLEP.S91125
- Schmidt M, Schmidt SAJ, Adelborg K, et al. The Danish health care system and epidemiological research: from health care contacts to database records. *Clin Epidemiol.* 2019;11:563–591. doi:10.2147/CLEP.S179083
- Benchimol EI, Smeeth L, Guttmann A, et al. The REporting of studies Conducted using Observational Routinely-collected health Data (RECORD) statement. *PLoS Med.* 2015;12(10):e1001885. doi:10.1371/journal.pmed.1001885
- Hansen DL, Overgaard UM, Pedersen L, Frederiksen H. Positive predictive value of diagnosis coding for hemolytic anemias in the Danish National Patient Register. *Clin Epidemiol.* 2016;8:241–252. doi:10.2147/CLEP.S93643
- Hansen DL, Møller S, Andersen K, Gaist D, Frederiksen H. Evans syndrome in adults - incidence, prevalence, and survival in a nationwide cohort. *Am J Hematol.* 2019;94(10):1081–1090. doi:10.1002/ajh.25574
- Mannering N, Hansen DL, Frederiksen H. Evans syndrome in children below 13 years of age – a nationwide population-based cohort study. *PLoS One.* 2020;15(4):e0231284. doi:10.1371/journal.pone.0231284
- Stata Statistical Software: release 15 [computer program]. College Station, TX: StataCorp LLC; 2017.
- Skov T, Deddens J, Petersen MR, Endahl L. Prevalence proportion ratios: estimation and hypothesis testing. *Int J Epidemiol.* 1998;27(1):91–95. doi:10.1093/ije/27.1.91
- StataCorp. *Stata Base Referecen Manual Release 15.* Stata Press; 2017.
- Statistics Denmark. Statbank; 2018. Available from: <https://www.statbank.dk/>. Accessed May 19, 2019.
- Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med.* 2002;347(12):911–920. doi:10.1056/NEJMra020100
- Lohi S, Mustalahti K, Kaukinen K, et al. Increasing prevalence of coeliac disease over time. *Aliment Pharmacol Ther.* 2007;26(9):1217–1225. doi:10.1111/j.1365-2036.2007.03502.x
- Norgard BM, Nielsen J, Fonager K, Kjeldsen J, Jacobsen BA, Qvist N. The incidence of ulcerative colitis (1995–2011) and Crohn's disease (1995–2012) - based on nationwide Danish registry data. *J Crohns Colitis.* 2014;8(10):1274–1280. doi:10.1016/j.crohns.2014.03.006
- Lastrup H, Voss A, Green A, Junker P. Occurrence of systemic lupus erythematosus in a Danish community: an 8-year prospective study. *Scand J Rheumatol.* 2009;38(2):128–132. doi:10.1080/03009740802419073
- Pedersen JK, Svendsen AJ, Horslev-Petersen K. Incidence of Rheumatoid Arthritis in the Southern part of Denmark from 1995 to 2001. *Open Rheumatol J.* 2007;1:18–23. doi:10.2174/1874312900701010018
- Andersson TM, Engholm G, Pukkala E, et al. Avoidable cancers in the Nordic countries-The impact of alcohol consumption. *Eur J Cancer.* 2018;103:299–307. doi:10.1016/j.ejca.2018.03.027
- Ocias LF, Larsen TS, Vestergaard H, Friis LS, Abildgaard N, Frederiksen H. Trends in hematological cancer in the elderly in Denmark, 1980–2012. *Acta Oncol (Madr).* 2016;55(sup1):98–107. doi:10.3109/0284186X.2015.1115124
- Bylsma LC, Gulbech Ordning A, Rosenthal A, et al. Occurrence, thromboembolic risk, and mortality in Danish patients with cold agglutinin disease. *Blood Adv.* 2019;3(20):2980–2985. doi:10.1182/bloodadvances.2019000476
- Hadnagy C. Age-wise distribution of idiopathic cold agglutinin disease. *Z Gerontol.* 1993;26(3):199–201.
- Luzzatto L, Risitano AM. Advances in understanding the pathogenesis of acquired aplastic anaemia. *Br J Haematol.* 2018;182(6):758–776. doi:10.1111/bjh.15443
- Gulbis B, Eleftheriou A, Angastiniotis M, et al. Epidemiology of rare anaemias in Europe. *Adv Exp Med Biol.* 2010;686:375–396.
- Gupta R, Pandey P, Choudhry R, et al. A prospective comparison of four techniques for diagnosis of paroxysmal nocturnal hemoglobinuria. *Int J Lab Hematol.* 2007;29(2):119–126. doi:10.1111/j.1751-553X.2006.00838.x

34. Lima M. Laboratory studies for paroxysmal nocturnal hemoglobinuria, with emphasis on flow cytometry. *Pract Lab Med.* **2020**;20: e00158. doi:10.1016/j.plabm.2020.e00158
35. Hillmen P, Hall C, Marsh JC, et al. Effect of eculizumab on hemolysis and transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria. *N Engl J Med.* **2004**;350(6):552–559. doi:10.1056/NEJMoa031688
36. Anderson IB, Sorensen TI, Prener A. Increase in incidence of disease due to diagnostic drift: primary liver cancer in Denmark, 1943–85. *BMJ (Clinical Research Ed).* **1991**;302(6774):437–440. doi:10.1136/bmj.302.6774.437
37. Arndt PA. Drug-induced immune hemolytic anemia: the last 30 years of changes. *Immunohematology.* **2014**;30(2):44–54.
38. Bass GF, Tuscano ET, Tuscano JM. Diagnosis and classification of autoimmune hemolytic anemia. *Autoimmun Rev.* **2014**;13(4–5):560–564. doi:10.1016/j.autrev.2013.11.010
39. Hill QA, Hill A, Berentsen S. Defining autoimmune hemolytic anemia: a systematic review of the terminology used for diagnosis and treatment. *Blood Adv.* **2019**;3(12):1897–1906. doi:10.1182/bloodadvances.2019000036
40. Jager U, Barcellini W, Broome CM, et al. Diagnosis and treatment of autoimmune hemolytic anemia in adults: recommendations from the First International Consensus Meeting. *Blood Rev.* **2019**; 100648. doi:10.1016/j.blre.2019.100648
41. Hill A, Hill QA. Autoimmune hemolytic anemia. *Hematology Am Soc Hematol Educ Program.* **2018**;2018(1):382–389. doi:10.1182/asheducation-2018.1.382

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