RESEARCH LETTER

blood advances

TO THE EDITOR:

Detrimental effects of sickle cell disease and hydroxycarbamide on ovarian reserve but uncertain impact on fertility

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We read with interest the recent report by Pecker et al.[1] Concomitantly with their work, we studied the ovarian reserve in women with severe sickle cell disease. Our objective was to characterize the impact of sickle cell disease and its treatments on the ovarian reserve and fertility in this context. Our findings were generally in line with those of Pecker et al but extended the latter's conclusions and shed new light on the physiopathology of diminished ovarian reserve in sickle cell disease.

The fertility of women with sickle cell disease has not been extensively explored, and the possible negative impact of the disease and/or its treatments (particularly hydroxycarbamide [HC]) on ovarian reserve is subject to debate. Two studies by Pecker et al demonstrated that HC treatment of patients with sickle cell disease was independently associated with lower anti-Müllerian hormone (AMH) levels, a validated biomarker of ovarian reserve.[1,2] Pecker et al showed that sickle cell disease may exacerbate the age-related decrease in AMH levels and, perhaps, ovarian aging.[2,3] Moreover, retrospective study by Kopeika et al highlighted a diminished ovarian reserve in women with sickle cell disease (n = 50), relative to age-matched control patients (n = 73).[4]

We prospectively analyzed the serum AMH level in a cohort of women with sickle cell disease whose disease characteristics, treatments (HC or transfusion), and maternal/pregnancy status were precisely known. Furthermore, to evaluate the potential detrimental effect of sickle cell disease per se, we compared the patients' AMH levels with those in age-matched controls and with age-class AMH reference values. The control population consisted of female patients who were free of sickle cell disease with normal-for-age AMH values visiting our institution's reproductive medicine unit for male, tubal, or unexplained infertility.

The study was performed at Necker Hospital (Paris, France) between 10 February and 6 April 2021, sponsored by the French government's Labex GR-Ex consortium (NCT03541525), and performed in accordance with regulatory requirements. All nonpregnant female patients with sickle cell disease aged between 16 and 40 years who gave their informed consent were eligible for inclusion. We used an AMH cut-off value of 1.2 ng/mL (as recommended by the Poseidon group) to define diminished ovarian reserve.[5] Our multivariate linear regression analysis was adjusted for age, HC exposure, vaso-occlusive events, proven hemochromatosis, acute chest syndrome, genotype, and transfusions.[6]

In total, 65 women with sickle cell disease (median age, 25 years [range, 16-40 years]) were included in the study (Table 1); 33 of 65 patients were receiving HC at the time of sample collection (forming the HC group), and 32 patients were not (forming the non-HC group). Of the 32 patients in the non-HC

Submitted 31 August 2022; accepted 17 March 2023; prepublished online on *Blood Advances* First Edition 29 March 2023; final version published online 24 August 2023. https://doi.org/10.1182/bloodadvances.2022008859.

Data are available upon request to the corresponding author, Laure Joseph (laure.joseph@aphp.fr).

The full-text version of this article contains a data supplement.

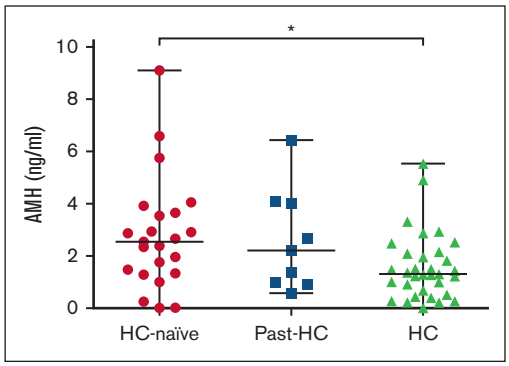

Figure 1. Serum AMH levels in women with sickle cell disease who had never taken HC (the HC-naive group), in those having taken HC in the past but who had discontinued the drug at least 12 months before sample collection (the past-HC group), and in those who were taking HC when the sample was collected (the HC group). The AMH level was significantly lower in the HC group than in the other 2 groups. There was no significant difference in AMH level between the HC-naive and past-HC groups. *$P = .035$ in a Kruskal-Wallis with Dunn posttest (expressed as the median [range]).

group, 9 had received HC at some point in the past (the past-HC group) (median duration, 2455 days [range, 1133-3925 days]) but had discontinued the drug at least 1 year before the sample collection (median wash-out period, 1436 days [range, 629-5157 days]). The median AMH level was significantly lower in the HC group than in the non-HC group (1.31 vs 2.47 ng/mL, respectively; $P = .01$; Table 1). Interestingly, there was no difference in AMH levels between patients in the past-HC group and those in the HC-naive group (2.21 ng/mL [range, 0.58-6.44 ng/mL] vs 2.55 ng/mL [range, 0.01-9.10 ng/mL], respectively; Figure 1). We did not find a correlation between the AMH level and the total cumulative dose of HC ($r = 0.0857$; $P = .7353$). Patients with vaso-occlusive events or proven hepatic iron overload during the year preceding study inclusion showed much the same AMH levels as patients not meeting these criteria ($r = 0.1265$; $P = .5213$, using Spearman test). We noted a significant positive correlation between the duration of transfusion and the AMH level ($n = 41$; $r = 0.3251$; $P = .0381$), but this effect was not significant in the non-HC group ($n = 22$; $r = 0.4061$; $P = .0607$; supplemental Figure 1). The frequency of diminished ovarian reserve (as defined by the AMH cut-off value) was higher (albeit not significantly) in the HC group than in the non-HC group (Table 1).

AMH levels were significantly lower in women with sickle cell disease than in age-matched control women (1.51 vs 3.4 ng/mL, respectively; $P < .0001$), even though the sickle cell disease group was slightly younger (median patient age, 25.0 years vs 25.7 years for the controls; $P = .024$; Table 1). Furthermore, the prevalence of diminished ovarian reserve was higher in the patients than in controls (29% vs 7%, respectively; $P = .011$; Table 1). For each age-class, the proportion of women with low AMH values was significantly higher in the sickle cell disease group (regardless of HC exposure) than in the controls (Table 1). As expected, a multivariate analysis confirmed that HC exposure was associated with a lower than normal AMH concentration ($r = -0.850$ [-1.60;-0.0930]; $P = .043$; supplemental Table 1). The other variables studied were not significantly associated with the AMH level in our population with sickle cell disease.

Our study of women with severe sickle cell disease showed that the AMH level was significantly lower among individuals taking HC than among nonexposed individuals. This confirmed the detrimental effect of HC on the reserve of growing follicles, as suggested in the results by Pecker et al.[1,2] We did not observe a dose-effect relationship between HC and the AMH levels, perhaps because we rarely prescribed the maximum tolerated dose of HC. To our knowledge, our study is the first to have observed similar AMH levels in woman previously exposed to HC and in those who are naive to HC treatment; it is possible that the AMH level normalizes (at least partially) after discontinuation of HC and that primordial follicles can survive the drug's toxic effects. However, the lack of long-term data on treatment compliance limits this supposition. This hypothesis is further supported by histologic data on the ovary in HC-treated mice; growing follicles were damaged, but the number of primordial follicles was similar to that observed in untreated controls.[7] However, the latter results in mice should be interpreted with caution because the sample size was small and should be confirmed in larger series.

A new important finding was the positive effect of transfusion on the AMH level. The association was not significant in individuals in the non-HC group, although the small size of this subgroup probably accounted for the lack of statistical significance. This effect of transfusion has been described for spermatogenesis in men with sickle cell disease and might be linked to better disease control.[8] The iron overload induced by regular transfusions or disease in sickle cell disease differs from that observed in thalassemia. In our cohort, iron overload was limited to the liver and did not appear to influence the AMH level.[9]

As suggested by Garba et al for patients who are less severely affected, we found that sickle cell disease per se had a detrimental effect: women in the non-HC group had a significantly lower ovarian reserve than the control population and relative to age-adjusted reference values.[10] The most crucial finding was the higher prevalence of diminished ovarian reserve in women with sickle cell disease (including younger women), which exposes the patient to a relative shorter lifespan, especially in a population with delayed puberty.[4,11] However, it is well known that AMH levels do not reflect the likelihood of natural conception.[12] In our cohort, 30 of 65 patients (including 15 in the HC group) had been pregnant at least once, which is reassuring (Table 1). Because of social, economic, and cultural factors, the median patient age at first pregnancy was lower in our cohort (24.0 years) than in the general population (28.8 years)[13]; this difference in age might have facilitated pregnancy. Furthermore, the ability of the AMH level to predict the time to menopause has not been unambiguously established and requires further investigation.[14]

This study's limitations include a lack of patients treated with the maximum tolerated dose of hydroxyurea and a lack of patients treated since birth. Although the women with sickle cell disease were younger than the control subjects, the intergroup difference in AMH levels (ie, with especially low levels in the former group) was even greater than expected. Furthermore, we lacked data on the age at puberty. The incidence of miscarriage was not reported; this would be an interesting variable for further study, because low AMH levels are associated with a higher risk of abortion.[15]

Table 1. Characteristics (notably AMH levels) of the patients with sickle cell disease as a function of HC exposure, together with control values and age-adjusted reference values

	Control patients (n = 130)	Patients with SCD (n = 65)	*P* value	Patients in the non-HC group (n = 32)	Patients in the HC group (n = 33)	*P* value
Age (y), median [range]	25.7 [20.5-40.2]	25.0 [16.0-40.0]	.024	25.0 [18.0-37.0]	24.0 [16.0-40.0]	.567
BMI (kg/m^2), median [range]	23.3 [17.3-37.6]	22.3 [17.1-39.6]	.2983	22.3 [17.6-33.7]	22.0 [17.1-39.6]	.9538
SCD genotypes, n (%)						
SS	–	51 (78)	N/A	24 (75)	27 (81)	.5580
SC	–	8 (12)	N/A	7 (22)	1 (3)	N/A
Sβ0	–	3 (5)	N/A	0 (0)	3 (9)	N/A
Sβ$^+$	–	3 (5)	N/A	1 (3)	2 (6)	N/A
Disease complications, n (%)	–					
Osteonecrosis	–	6 (9)	N/A	1 (3)	5 (15)	.1968
Retinopathy	–	11 (17)	N/A	5 (16)	6 (18)	1.000
Nephropathy	–	7 (11)	N/A	3 (9)	4 (12)	N/A
Cerebral vasculopathy	–	29 (45)	N/A	15 (46)	14 (42)	.8050
Liver hemochromatosis	–	28 (43)	N/A	13 (40)	15 (45)	.8036
Acute chest syndrome	–	29 (45)	N/A	9 (28)	20 (60)	.1778
Vaso-occlusive episode (at least 1 in the last 12 months)	–	40 (62)	N/A	17 (53)	23 (69)	1.000
Biochemical and hematologic features						
Hemoglobin (g/dL)	–	9.2 [7.2-12.2]	N/A	9.5 [7.2-12.2]	9.0 [7.2-11.8]	.2089
LDH (UI/L), median [range]	–	348 [27-770]	N/A	358 [27-770]	348 [193-531]	.2403
Reticulocyte count (G/L), median [range]	–	223 [49-674]	N/A	225 [78-674]	223 [49-611]	.3207
MCV (fl), median [range]	–	84 [59-122]	N/A	82 [59-93]	86 [65-122]	.0075
HbF (%), median [range]	–	2.3 [0.4-29.1]	N/A	1.6 [0.4-6.0]	3.7 [0.5-29.1]	.0008
HC						
Dose (mg/kg per day), median [range]	–	18.99 [7.14-36.11]	N/A	–	18.99 [7.14-36.11]	N/A
Duration (day), median [range]	–	2491 [140 –7154]	N/A	–	2491 [140 –7154]	N/A
Transfusion program, n (%)	–	41 (63)	N/A	22 (69)	19 (58)	.8155
Motherhood, n (%)	–	30 (46)	N/A	15 (46)	15 (45)	1.000
AMH (ng/mL), median [range]	3.40 [0.60-17.60]	1.51 [0.01-9.10]	< .0001	2.47 [0.01-9.10]	1.31 [0.01-5.54]	.01
DOR, n (%)	9 (7)	19 (29)	.011	7 (22)	12 (36)	.27

Low values for age are AMH values below the reference values established for an age class. Diminished ovarian reserve is defined as an AMH level below 1.2 ng/mL.

Controls and patients were matched as 2:1 for age. The matched control population consisted of patients who were free of sickle cell disease with healthy AMH values for age, attending our institution's reproductive medicine unit for male, tubal, or unexplained infertility. Matching according to the age was carried out by staff who were aware of the matching criteria but otherwise blinded to the AMH results.

We used the Mann-Whitney or Kruskal-Wallis test with Dunn posttest to analyze quantitative variables (expressed as the median [range]) and Fisher exact test to analyze categorical variables.

BMI, body mass index; DOR, diminished ovarian reserve; HbF, fetal hemoglobin; LDH, lactate dehydrogenase; MCV, mean corpuscular volume; SCD, sickle cell disease.

Table 1 (continued)

	Control patients (n = 130)			Patients with SCD (n = 65)				Patients in the non-HC group (n = 32)				Patients in the HC group (n = 33)			
Age (y)	Samples (n)	AMH (ng/mL) Median [range]	Percent of low values for age	Samples (n)	AMH (ng/mL) Median [range]	Percent of low values for age	*P* value (Fisher exact test)	Samples (n)	AMH (ng/mL) Median [range]	Percent of low values for age	*P* value (Fisher exact test)	Samples (n)	AMH (ng/mL) Median [range]	Percent of low values for age	*P* value (Fisher exact test)
[16-19]	0	–	N/A	15	2.53 [1.30-5.75]	–	N/A	6	3.10 [1.39-5.75]	–	N/A	9	2.10 [1.30-2.94]	–	N/A
[20-24]	32	3.97 [1.12-11.10]	3.125	16	2.13 [1.00-9.10]	43.75	<.0001	8	3.47 [1.29-9.10]	25.00	< .0001	8	1.49 [1.00-4.91]	62.50	<.0001
[25-29]	53	4.00 [0.61-17.70]	1.89	11	2.35 [0.27-4.07]	27.27	<.0001	9	2.55 [1.00-4.07]	11.10	.005	2	0.64 [0.27-1.01]	100.00	N/A
[30-34]	24	2.55 [0.60-13.46]	4.17	12	1.19 [0.01-3.65]	33.33	<.0001	7	0.91 [0.01-3.65]	42.90	< .0001	5	1.44 [0.45-2.88]	20.00	N/A
[35-39]	17	2.26 [0.92-4.98]	0	9	0.52 [0.02-2.39]	44.44	<.0001	2	1.21 [0.02-2.39]	50	N/A	7	0.52 [0.22-1.24]	42.86	.0007
40	4	2.14 [0.90-3.08]	N/A	2	0.21 [0.01-0.40]	–	N/A	0	–	–	N/A	2	0.21 [0.01-0.40]	–	N/A

Low values for age are AMH values below the reference values established for an age class. Diminished ovarian reserve is defined as an AMH level below 1.2 ng/mL.
Controls and patients were matched as 2:1 for age. The matched control population consisted of patients who were free of sickle cell disease with healthy AMH values for age, attending our institution's reproductive medicine unit for male, tubal, or unexplained infertility. Matching according to the age was carried out by staff who were aware of the matching criteria but otherwise blinded to the AMH results.
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Furthermore, it would be useful to carry out a longitudinal study of women with sickle cell disease, in order to characterize the change over time in AMH, the duration of their reproductive window, and the impact of the disease and its treatments.

As recently proposed by Pecker et al, our results strongly suggest that the medical care of women with sickle cell disease should include a fertility consultation, an evaluation of ovarian reserve, and a discussion of future fertility in individuals with a low AMH value, particularly if they wish to postpone childbearing.[1] In any case, prospective studies are needed to better understand the underlying factors that act on folliculogenesis in women with sickle cell disease (whether treated with HC or not), assess the impact of diminished ovarian reserve on childbearing, and determine the management of the diminished ovarian reserve in this population.

Contribution: L.J. designed the study, included patients, analyzed data, and wrote the manuscript; V.B.-L. designed the study, analyzed data; and wrote the manuscript; S.M. performed statistical analysis and collected data; D.B. performed biochemical analysis; C.P. and P.S. designed the study, analyzed data, and corrected the manuscript; J.B.A. included patients and corrected the manuscript; B.M. included patients; M.C. designed the study and corrected the manuscript; and all authors have read and approved the final manuscript.

Conflict-of-interest disclosure: The authors declare competing financial interests.

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