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Are there anamnestic risk factors for iron deficiency in pregnancy? Results from a feasibility study

Abstract: The conditions of iron deficiency are highly incident in pregnancy with elevated risks for preterm birth and low birth weight. In our recent study, we found 6% of participants having anemia, whereas between 39% and 47% showed iron deficiency without anemia. In many countries in prenatal care solely hemoglobin (Hb) measurement is applied. For the gynecologists till date there is no indication to determine other markers (e.g., serumferritin). As iron deficiency results from an imbalance between intake and loss of iron, our aim was to find out if the risk of iron deficiency conditions can be estimated by a diet history protocol as well as questionnaires to find about iron loss. We found that the risk of having iron deficiency in upper gestational week (>=21) increased by a factor of five. Thus, additional diagnostics should be done in this group by now. Using the questionnaire as a screening instrument, we further estimated the probability of disease in terms of a positive likelihood ratio (LR+). The positive LR for the group below 21th week of gestation is 1.9 thus, increasing the post-test probability to 52% from 36% as before. Further research based on higher sample sizes will show if the ratios can be increased further.

Keywords: Anemia; iron deficiency; iron intake; iron losses; likelihood ratio; risk factors.

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Introduction

In Germany, as in many other countries, according to given guidelines iron diagnostic in pregnancy is limited to hemoglobin measurement while additional markers are not usually applied. Thus, only pregnant women with anemia will be detected and subsequently treated with supplements at appropriate dosages prescribed by the attending obstetrician. Iron deficiency without anemia is not under observation and supplementation is left to the pregnant women with respect to onset as well as the selection of preparations concerning iron content. In most over the counter preparations iron content is however too low to stop deteriorating iron stores or even to cure condition of iron deficiency. To date, health care authorities are not recommending the broad application of additional iron markers in prenatal care which at best should be applied in early pregnancy in order to detect iron deficiency without anemia timely. Thus, the question arises if there are anamnestic risk factors for iron deficiency. This would allow the concentration of additional diagnostic measurements initially on this target population.

Theoretical background and aims of the study

Depletion of iron stores are resulting due to an imbalance among iron requirements, iron intake, and iron losses. From a theoretical point of view, the probability of iron deficiency should increase when iron intake is insufficient and iron losses are high and *vice versa*. Both intake and losses can be determined in surveys with questionnaires. Besides an actual and mutual determination of the prevalences of iron deficiency conditions – results are published separately – the study had two aims: (i) to develop a questionnaire to determine iron losses in addition to nutritional iron intake measured by a given diet history questionnaire (7-day protocol of the German Society of Nutrition) and (ii) to investigate if diagnosed iron deficiency conditions and reported nutritional iron intake as

well as iron losses are associated in the questionnaire. If so, there would be a chance to predict the probability of iron deficiency in individuals.

Questionnaire

In addition to the given diet history tool operationalizing iron intake [Question number (Qn): 78], questions to determine iron losses were developed (Qn: 52–59) dealing with duration and strength of former menstruation, gingival bleeding, blood donation, operations, reported iron deficiency or anemia in the last 4 weeks or 12 months, etc. We also allowed for some symptoms that may be a sign of iron deficiency (e.g., brittle nails) (Qn: 37).[1]

Diet history protocols are standard instruments in nutritional counselling and often also used in epidemiological research. "They are considered to be the gold standard of self-reporting techniques and have been used to validate other methods [1, p. 8]". From a more recent study we, however, know that iron intake is a good predictor of incorporated iron stores in men in contrast to women [Dudenhausen et al., unpublished manuscript]. This can be explained by the fact that in women there are often enormous blood and respective iron losses especially due to menstruation or possible preceding birth. This was the rationale for the development of the questionnaire concerning iron losses. This questionnaire is an in-house development which was not yet tested for validity or reliability and we are interested especially in proposals how to perform a validity test.

Study design and study population, ethical board approval

A total of 20 gynecologists were asked to participate in our study which was carried out from spring to late summer 2013 in the city of Berlin. A total of 200 pregnant women in the first and second trimester (up to 28 weeks of gestation) should be enrolled. Sample size was derived under financial and statistical considerations. Based on the given European prevalence data [2], we assumed that about 50% will have anemia or iron deficiency without anemia. The comparison of questionnaire data between participants with and without diagnosed iron deficiency or anemia should be done by *t*-test statistics and the calculation of odds ratios. By the calculated sample size both indicators would provide significant results by group percent differences of approximately 15%.

Enrollment was done as follows:

- participants (with written confirmation) received the BabyCare Program [3] and were asked to fill in the questionnaire reliable, complete, and accurate and to bring it back to the practice at the next prenatal care visit
- when the questionnaire was filled in completely (controlled by the practice staff), blood samples were taken and sent to one co-operating laboratory
- laboratory results were transmitted back to the practices
- in case of anemia or iron deficiency without anemia the participants received an iron supplement (100 mg/day) with adherence instructions
- in this case second blood samples were taken approximately 4 weeks later

All procedures and data had to be documented on a standardized case report form. The study obtained approval from the responsible Ethics Committee of Charité University Medicine Berlin.

Laboratory parameters, definitions, and classifications

The samples were analyzed for hemoglobin (Hb), C-reactive protein (CRP), transferrin saturation (TFS), and serum ferritin (see Table 1). Because serum ferritin may yield false negative results in the presence of infection or chronic inflammation, CRP was analyzed additionally and in participants with CRP >5 mg/L indicating infection or inflammation, TFS was used for the interpretation and classification of results.

Table 1 Laboratory parameters, threshold values, and classifications.

	Parameters	Units		Results	Groups
1	Hemoglobin	g/dL	<11	Anemia	A
2	Serum-ferritin	ng/mL	≥30	No deficiency	O
			<30	Deficiency	B
3	C-reactive protein	mg/L	≤5	No inflammation	
			>5	Inflammation[a]	
4	Transferin saturation	%	≥20	No deficiency	O
			<20	Deficiency	B

Hb=Hemoglobin, CRP=C-reactive protein, TFS=transferrin saturation, A=anemia, B=iron deficiency, O=neither/nor.

[a]Serum ferritin value is not conclusive. TFS value was used instead.

1 You can view the questionnaire under: https://www.baby-care.de/downloads/de-de/fragebogen_internet.pdf

In our study, the soluble transferrin receptor (sTfR), which is increasingly advocated, was not assessed for reasons of costs. In accordance with many actual clinical and epidemiological studies, Hb, serum ferritin and transferrin, as well as CRP were assessed, the latter to control for inflammation. We used different methods to deal with the problem of inflammatory cases, which led to quite consistent prevalence estimates for our study population. However, as substantial misclassification will certainly influence the likelihood estimates, we finally compared the likelihood estimates for the whole sample with the estimates for a subsample where CRP-positive cases were excluded. Based on remaining n=96 cases, the positive likelihood ratio (LR) is 1.96 compared to 1.90 for all n=148 cases (see below). By this we conclude that there is no substantial misclassification.

Based on 193 participants finally enrolled with corresponding questionnaire as well as laboratory data study, we found 6% of participants having anemia, whereas between 39% and 47% showed iron deficiency without anemia. This prevalence range is due to different methods used to deal with inflammatory cases and the lower range value certainly will be an underestimation. Overall, iron deficiency is significantly increasing by gestational age from 41.3% (≤20) to 66.7% (≥21) (P=0.01).

Risk factors of iron deficiency and anemia

The empirical aim of the study was to investigate if diagnosed iron deficiency/anemia and reported nutritional iron intake as well as blood losses are associated in the questionnaire. To do so, in the first step we compared the groups with and without diagnosed iron deficiency/anemia for the variables indicating iron intake and iron losses (see Table 2) calculating odds ratios. We can easily see that nutritional iron intake does not discriminate between the groups. Although an intake below 50% of the recommended amount of 30 mg iron daily in pregnancy is higher in the deficiency group yielding an odds ratio of 1.4, this however is far from being significant on the sample size given. Contrary to our hypothesis, there was also no association between reported and diagnosed anemia or iron deficiency. We can, however, clearly recognize that menstruation characteristics as well as week of gestation are highly associated with iron deficiency. The probability of iron deficiency or anemia increases when (i) week of gestation is ≥21; (ii) menstrual blood flow is 6 days or longer (borderline significance); and (iii) tampon type used has high absorbency indicated by four to six water drops at any point during menstruation but especially in the middle of the period. When both menstrual blood flow is long and a tampon with high absorbency was used at least once in the cycle the probability for iron deficiency is threefold higher.

In the second step, we investigated if the respective questions and variables can be used as a screening model or diagnostic test looking at the pre- and post-test probabilities and the LRs. To do so, first of all the original codes of the variables were substituted by the odds ratios calculated. To give an example: there are six different tampons types which were originally coded from 1 (one drop) to 6 (six drops). The codes 4–6 were now substituted by the

Table 2 Differences in the frequency distribution of variables between groups with and without diagnosed iron deficiency and odds ratios.

	Total	Iron deficiency or anemia	None	Odds ratio	Confidence Interval 95%	
n	**193**	**88**	**105**			
≥21 weeks of gestation	14.6%	24.2%	6.1%	4.95[a]	2.02	12.14
Menstrual blood flow ≥6 days and tampon type at least once ≥4	25.9%	37.5%	15.8%	3.19[a]	1.63	6.23
At least once tampon type ≥4	54.3%	66.7%	43.6%	2.59[a]	1.44	4.67
Tampon type middle of period 4 to 6	42.6%	52.9%	33.7%	2.21[a]	1.23	3.97
Intensity of the menstrual blood flow (middle or strong)	85.5%	89.9%	81.7%	1.99	0.86	4.60
Tampon type middle of period end of period 4 to 6	23.4%	29.9%	17.8%	1.97	1.00	3.88
Menstrual blood flow ≥6 days or tampon type at least once ≥4	69.8%	77.3%	63.4%	1.97[a]	1.04	3.72
Tampon type beginning of period 4 to 6	38.3%	46.0%	31.7%	1.84[a]	1.02	3.32
Menstrual blood flow ≥6 days	41.6%	48.9%	35.3%	1.75	0.98	3.13
Brittle nails	22.6%	27.1%	18.4%	1.64	0.82	3.26
Iron intake <50% below recommended intake (30 mg in pregnant)	75.1%	78.7%	72.1%	1.42	0.73	2.76
Iron intake <90% below recommended intake (15 mg before pregnant)	63.2%	66.3%	60.6%	1.28	0.71	2.31
Iron intake <70% below recommended intake (15 mg before pregnant)	31.6%	33.7%	29.8%	1.20	0.65	2.20

[a]Significant OR (α=0.05).

odds ratio and the codes 1–3 by its complement, in the case of beginning period equal to 1.84 (see Table 2) and (1/1.84=0.54), respectively.

This procedure was done for all significant variables of the table and in addition, though not yet significant based on the sample size given, the intensity of menstrual blood as well as iron intake <50% below recommended intake in pregnancy. For this analysis we had to exclude six participants due to missing item values not correctable. As week of gestation obviously is a predictive factor for iron deficiency we restricted this analysis to pregnant women below 21 weeks of gestation leading to 158 cases. Furthermore, 10 cases with anemia already diagnosed by Hb diagnostics had to be excluded from this analysis. So, finally a sample size of 148 remained. In this group, the prevalence of non-anemic iron deficiency now is 36.5%.

For each participant, the odds values of the variables were summarized yielding a distribution shown in Table 3. The group of participants with iron deficiency has significant higher mean (P=0.003) and median values (P<0.001). In the next step, a threshold value had to be derived which would be optimal with respect to screening criteria. To do so, these criteria were calculated for different cut-off levels (see Table 4). Screening tests are assessed using sensitivity, specificity, and predictive values in a population wide approach. But to estimate the probability of disease in an individual patient the positive or negative LR (LR+, LR-) are the appropriate indicators [4]. With both a pretest probability of a disease given, which normally is the prevalence, hence 36.5%, a post-test probability can be calculated and illustrated by Fagan's Nomogram.

"The Fagan's Nomogram is the simplest of the Bayes' theorem calculators to help practitioners determine the probability of a patient truly having a condition of interest given a particular test result" [5, p. 128]. If the LR of the test result is greater than one and very large, the evidence provided by the test result strongly supports the presence of the condition. However, if the LR of the test result is smaller than one and very close to zero, the evidence provided by the test result strongly supports the absence of the condition. The optimal cut-off point is calculated as 7.8 according to the formula suggested (see Table 4). By this, the post-test probability is considerably increasing to 52% as is shown in Figure 1.

Table 3 Distribution properties.

	n	%	Mean	SD	Median
Total	148	100.0	7.6	2.2	7.6
No iron deficiency	94	63.5	7.2	2.0	6.8
Iron deficiency	54	36.5	8.3	2.2	8.4
			P=0.003		P<0.001

Summary and discussion

In our study more than 40% of participants show iron deficiency without anemia. They are at risk of developing anemia in ongoing pregnancy when there is no or insufficient supplementation. This risk group cannot be detected by Hb measurements usually applied. According to our additional findings, the probability of developing acute iron deficiency or anemia is significantly increased in upper weeks of gestation (>=21th) and also with several menstrual characteristics. With iron intake there are no so far only non-significant associations. Probably iron intake is generally too low. Additional iron diagnostics should therefore be offered to women above 20 weeks of pregnancy.

However, even in earlier pregnancy women with specific menstrual characteristics should be tested in this way because with these anamnestic informations the probability of detecting iron deficiency without anemia will increase on the average from 36% to 52%. So by screening the early forms of iron deficiency in time, targeted and physician-based supplemental recommendations can be given. This may also allow for lower dosages with the possibility of increasing adherence.

Our study has some limitations which are due to poor evidence and data in the planning phase. Although there were data available on the prevalence of iron deficiency conditions in several pregnant populations from Europe which could be used for sample size calculation, there was no evidence either on the distribution properties of the variables concerning iron losses or on the associations of both intake and loss variables with diagnosed iron deficiency conditions. Thus, the study must be characterized as a feasibility study. Though we assumed that iron deficiency conditions would be higher in upper gestational weeks, the noticeable increase from 21 to 28 weeks of gestation was not expected. The same stands for the high association of upper gestational week with iron deficiency conditions. As there is obviously no need for a screening instrument with respect to known gestational week, the screening model had to be restricted to the lower gestational age group leading, however, to a substantially reduced sample size. This

Table 4 Scores and results for screening criteria.

Results of the screening test	Iron status		
	Positive	Negative	
Positive	a	b	a+b
Negative	c	d	c+d
	a+c	b+d	a+b+c+d

						Optimal cut-off level				
	Cut-off levels	6.0	6.5	7.0	7.5	7.8	8.0	8.5	9.0	9.5
True positive cases	a	**45**	**40**	**38**	**38**	**36**	**34**	**25**	**25**	**16**
True negative cases	d	**30**	**41**	**52**	**57**	**61**	**62**	**71**	**71**	**81**
False positive cases	b	64	53	42	37	33	32	23	23	13
False negative cases	c	**9**	**14**	**16**	**16**	**18**	**20**	**29**	**29**	**38**
Total cases	a+b+c+d	148	148	148	148	148	148	148	148	148
Sensitivity	a/(a+c)	83%	74%	70%	70%	67%	63%	46%	46%	30%
Specificity	d/(b+d)	32%	44%	55%	61%	65%	66%	76%	76%	86%
1-Specificity	1−((d/(b+d))	68%	56%	45%	39%	35%	34%	24%	24%	14%
Prevalence	(a+c)/(a+b+c+d)	36%	36%	36%	36%	36%	36%	36%	36%	36%
Prop, of false positive cases	b/(a+b)	59%	57%	53%	49%	48%	48%	48%	48%	45%
Prop, of false negative cases	c/(c+d)	23%	25%	24%	22%	23%	24%	29%	29%	32%
Positive predictive value	a/(a+b)	41%	43%	48%	51%	52%	52%	52%	52%	55%
Negative predictive value	d/(c+d)	77%	75%	76%	78%	77%	76%	71%	71%	68%
Positive likelihood ratio	(a/(a+c))/((1−(d/b+d))	1.22	1.31	1.57	1.79	1.90	1.85	1.89	1.89	2.14
Negative likelihood ratio	(1−((a/a+c))/(d/b+d))	0.52	0.59	0.54	0.49	0.51	0.56	0.71	0.71	0.82
Optimal cut-off point: MIN!	(1−Sens)*(1−Sens)+ (1−Spez)*(1−Spez)	0.49	0.39	0.29	0.24	0.23	0.25	0.35	0.35	0.51

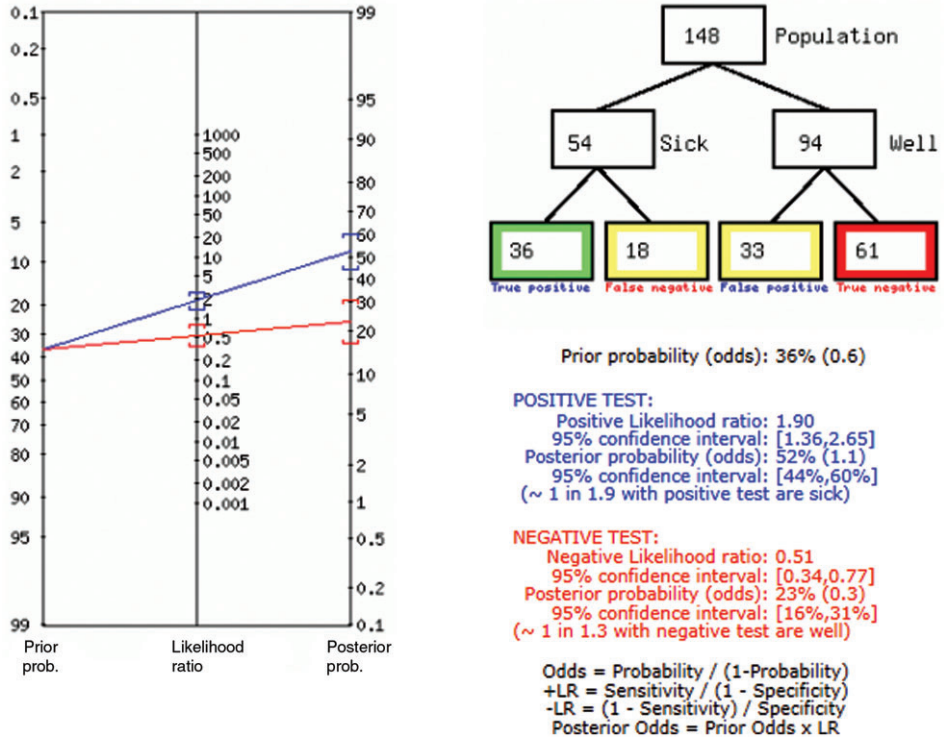

Figure 1 Fagans Nomogram (http://araw.mede.uic.edu/cgi-bin/testcalc.pl).

implies a number of not significant associations and also wide confidence intervals. A planned continuation of the study with approximately 500 cases in early pregnancy (≤20 week of gestation) will yield significant results by group percent differences of 5%. We will analyze if post-test probabilities can be increased and the predictions can be improved.

In ongoing pregnancy, iron deficiency without anemia will develop to anemia as well as normal iron conditions will lead to deficiency when iron intake is still insufficient. From an epidemiological perspective, the given prevalences of iron deficiency without anemia must be assessed as relatively high and the reluctance of health care authorities to extend the limits of Hb measurement is incomprehensible. Rationale medicine is based on timely and effective diagnostic measurements and targeted therapy which both is not the case momentarily.

Before additional iron diagnostics will become available by a revision of guidelines, obstetricians in prenatal care should be aware of their responsibility and recommend and carry out the additional laboratory examinations as suggested. The simple and requestable anamnestic informations may serve as an indication.

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