Review

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A review of the cut-off points for the diagnosis of vitamin B_{12} deficiency in the general population

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Abstract: Vitamin B_{12} deficit is one of the most common vitamin deficiencies. However, there is no consensus on the cut-off points for vitamin B_{12} and its co-markers, such as folate, holotranscobalamin, methylmalonic acid and homocysteine. In order to establish the state of the art about cut-off points used to determine vitamin B_{12} deficiency in the last decades, the database MEDLINE was used for searching studies published in adults between December 1992 and May 2014 (69 articles), using search terms like 'vitamin B_{12}', 'cobalamin', 'cut-off', 'deficiency' alone or in combinations. Broad ranges of cut-off points for vitamin B_{12} and its biomarkers were identified: vitamin B_{12} ranged between 100 pmol/L and 350 pmol/L, holotranscobalamin 20–50 pmol/L, methylmalonic acid 0.210–0.470 μmol/L, homocysteine 10–21.6 μmol/L, serum folate 3.7–15.9 nmol/L and red blood cell 124–397 nmol/L. For the majority of studies, the potential influence of age, analytical methods, gender and fortified food consumption was not taken in account when choosing cut-off values. This could explain the discrepancies between studies on vitamin B_{12} and folate deficiency prevalences. We conclude that there is inconsistency in the literature regarding vitamin B_{12} cut-offs. It would be necessary to establish different reference cut-offs according to age, considering the analytical methods used.

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Keywords: cut-offs; folate; homocysteine; methylmalonic acid; vitamin B_{12} deficiency.

Introduction

Vitamin B_{12} deficiency is still an important nutritional problem worldwide as subclinical deficiency affects well defined risk groups. Vitamin B_{12} levels decreases with age [1], which means that deficiency risk increases in parallel with age [2–4]. Therefore, subclinical deficiency is quite more prevalent in the elderly. Epidemiologic data shows that prevalence ranges from 6% to 40% [5]. However, at younger ages, a higher risk of developing vitamin B_{12} deficiency has been described among vegetarians [5, 6], patients with gastrointestinal disease [7], people with depression [8], people with high alcohol consumption [9], and people suffering from renal insufficiency [7]. The main manifestations of vitamin B_{12} deficiency are hematologic, neurologic and psychiatric disorders. Notably, vertigo, tiredness, malaise and cognitive impairment have been traditionally ascribed as 'normal aging' signs [10]. Most of these disorders and symptoms are usually seen as 'normal aging' signs. However, severe vitamin B_{12} deficiency causes an irreversible degeneration of the nervous system [11]. Thus, an early diagnosis and treatment of subclinical vitamin B_{12} deficiency is essential. In order to reach a more efficient diagnosis, a combination of several markers associated with vitamin B_{12} metabolism could be used in place of a single vitamin B_{12} measurement [5]. The most reliable markers are serum folate (sFolate), red blood cell folate (RBC folate), holotranscobalamin (HoloTC), methylmalonic acid (MMA) and homocysteine (Hcy).

One of the major problems when attempting to diagnose vitamin B_{12} deficiency is the choice of the cut-off values for each of the markers. For instance, due to the high variability of cut-off values across laboratories, vitamin B_{12} deficiency prevalence ranges from 3% for people aged ≥3 years to 67.6% for elderly (71–74 years) [12, 13]. This prevalence can increase up to 15.8% for institutionalized elderly, which presents a greater risk for

vitamin deficiencies than the free-living elderly [1]. Epidemiologic data by Andres et al. confirmed that 20% of the elderly present megaloblastic anemia in developed countries [14]. Finally, the different analytical methods used to measure vitamin B_{12} and its co-markers introduce an additional dispersion factor for cut-off setting.

The objective of this study is to provide a review of the published biomarkers and cut-off values for the diagnosis of vitamin B_{12} deficiency with respect to age, gender and methods of detection. The lack of consensus worldwide calls for a re-examination of the reference values currently employed to detect mild, moderate and severe forms of vitamin B_{12} deficiency.

Materials and methods

The electronic database MEDLINE (http://www.ncbi.nlm.nih.gov) was searched for studies published between December 1992 and May 2014. Terms 'vitamin B_{12}', 'cobalamin', 'cut-off', 'deficiency' as well as combinations out of these terms were entered in the database. In addition, references in relevant articles were also used to get further information.

Only articles containing vitamin B_{12} cut-offs were chosen. The rest of cut-offs (MMA, Hcy, HoloTC, sFolate and RBC folate) were collected when they were available. Additional data including analytical methods, geographic area, sample size and age were included if available, whereas health status of the population under study was not considered. Data on creatinine was also taken into account when authors gave this information to assess renal function. Articles were excluded if the cut-off for vitamin B_{12} was not included, or in case of a review, a systematic review or meta-analysis.

Results

A total of 69 articles were included in this review. Table 1 summarizes the main data in each study, ordered from lowest to highest cut-off points of plasma vitamin B_{12} concentration.

Nine studies involved a sample size ≤100 persons, 39 studies between 100 and 500, eight articles between 500 and 1000 and 13 with more than 1000 participants. The overall number of participants was 48,868 subjects. Forty-two studies specified the gender of the participants, 18 studies did not provide gender information and nine were only performed in one gender. In terms of age, 41 studies recruited subjects ≥60 years and 25 studies subjects ≤60 years. Three studies divided the population in different age groups [12, 13, 15] and only one depending on racial-ethnic group [12]. Forty studies were carried out in European countries, 20 in American countries, four in Asiatic countries, three in oceanic countries and two in Africa.

Vitamin B_{12}

The current standard clinical screening test for vitamin B_{12} deficiency is the determination of total vitamin B_{12} concentration in plasma or serum. Some authors considered deficiency when vitamin B_{12} concentration was below the low limit of indeterminate interval (grey zone), and others did not specify if they took the low limit of this indeterminate interval or the low limit of the reference range.

Cut-offs employed to define vitamin B_{12} ranged from 100 pmol/L [16, 17] to 350 pmol/L [18]. Thirteen authors chose 150 pmol/L to establish vitamin B_{12} deficiency and 12 reported a value of 148 pmol/L. Therefore, 37% of authors used nearly the same value.

Lindenbaum et al. chose a serum vitamin B_{12} of 258 pmol/L as a cut-off value based on high serum MMA concentrations in subjects with vitamin B_{12} values below this number [19]. Wolters et al. chose a cut-off value of 258 pmol/L for their study population of 178 senior females (60–70 years) [2]. They admitted that it might be too high, because the MMA concentrations in subjects in the second quartile (227–≤269 pmol/L) of serum vitamin B_{12} were not significantly different from those in subjects in the third (270–≤330 pmol/L) and fourth quartiles (<331 pmol/L). Rajan et al. suggested 221 pmol/L was a more appropriate cut-off [20]. Others recommend to restructure the normal range and to raise the vitamin B_{12} cut-off point for elderly to a much higher value than 220–260 pmol/L [21]. However, recently, Valente et al. used the lower limit of the 95% central reference interval (123 pmol/L) as established from their reference population [22].

Holotranscobalamin

Cut-offs for HoloTC ranged between 20 and 50 pmol/L. The lower one was established by Valente et al. [22] by using FPIA measurement and the higher one was set by Hvas and Nexo [23], Bor et al. [24] and Llody-Wright et al. [25] using CPB and ELISA analytical methods, respectively. The principal methodology run was RIA assay (65%) and the corresponding cut-off range was between 35 and 40 pmol/L. Only 20 authors considered HoloTC in their studies.

Table 1 Overview of different cut-offs used to define vitamin B_{12} deficiency in selected studies, sorted by the concentration of vitamin B_{12} cut-off.

No.	References	City (country)[a]	n (all; m, f)	Age, year	B_{12}[b], pmol/L	HoloTC[b], pmol/L	MMA[b], µmol/L	Hcy[b], µmol/L	sFolate[b], nmol/L	RBC folate[b], nmol/L	Creatinine[b], µmol/L
1	[16]	Wageningen (NL)	120	≥70	100		0.260				120
					CLIA		GC-MS				
2	[17]	Germany (DE)	202; 52, 150	81±6[g]	100		0.320				120
					ND		ND				
3	[15]	(BE, DE, NL)	1=99; 53, 46	1=young subpopulation 19–55	103		0.247	13.9	5.4		124
			2=64; 20, 44	2=healthy elderly people 65–88	RIA		GC-MS	GC-MS	RIA		
			3=286; 115, 171	3=hospitalized elderly 61–97							
4	[37]	13 centers (BE)	285; 105, 180	65–96[c]	103		0.247	13.9	5.4		
					RIA		GC-MS	GC-MS	RIA		
5	[38]	Skutskär (SE)	224; 94, 130	78 (77.2–78.9)[d]	115		0.370	15	3.7	275	
					RIA		ND	ND	RIA	ILA	
6	[39]	Toronto (CA)	711; 403, 308	58[e]	120			15		215	120
					CPB			HPLC		CPB	
7	[22]	Dublin (IE)	700; 210, 490	63–97	123	20	0.36	15	6.8	340	
					MBA	FPIA	GC-MS	FPIA	MBA	MBA	
8	[40]	Cardiff (GB)	49	<75	125	38	0.470	15			
					EIA	RIA	GC-MS	FPIA			
9	[41]	Sydney (AU)	2963	≥50	125 ND			15 ND	6.8 ND		
10	[24]	Copenhagen (DK)	98; 0, 98	41–75[c]	130	50	0.280	11.9			115 (f)
					CLIA	ELISA	GC-MS	FPIA			
11	[42]	Saarland (DE)	232; 79, 153	69 (19–102)[f]	132		0.271	15	6.4		106
					ND		ND	ND	ND		
12	[25]	Oxford (GB)	195; 195, 0	44 (18–78)[f]	135	50	0.280	12	6.8		
		London (GB)			EIA	ELISA	GC-MS	GC-MS	EIA		
13	[43]	Finland (FI)	2806; 1328, 1478	45–74[c]	138						
					CLIA						
14	[27]	Los Angeles (US)	591; 345, 246	>60	140		0.376	17.1 (m) 16.8 (f)	5.7		133 (m)
					RA		HPLC	HPLC	RIA		115 (f)
15	[44]	Perth (AU)	299	≥75	140			15			
					ND			ND			
16	[45]	Tel-Hashomer (IL)	1167	≥69	147		0.240	15	11		141
					ELISA		HPLC	RIA	ELISA		
17	[14]	Strasbourg (FR)	201; 57, 144	67±6[g]	148			13	6.8		120
					RIA			GC-MS	ND		
18	[46]	Florida (US)	359; 0, 359	20–30[c]	148	35		14			
					RIA	RIA		HPLC			
19	[47]	Gothenburg (SE)	209	76 (70–93)[h]	148		0.340	16	8.6		
					RIA		GC-MS	HPLC	RIA		
20	[48]	Sacramento (US)	1789; 751, 1038	≥60	148	35	0.350	13		363	124 (m)
					RIA	RIA	LC-MS	HPLC		CLIA	97 (f)

(**Table 1** Continued)

No.	References	City (country)[a]	n (all; m, f)	Age, year	B_{12}[b], pmol/L	HoloTC[b], pmol/L	MMA[b], µmol/L	Hcy[b], µmol/L	sFolate[b], nmol/L	RBC folate[b], nmol/L	Creatinine[b], µmol/L
21	[49]	United States (US)	1145	≥65	148		0.370				133 (m)
					RIA		GC-MS				115 (f)
22	[1]	Granada (ES)	218; 82, 136	79.2 (60–105)[f]	148	45	0.300	12	6	397	
					MEIA	RIA	GC-MS	FPIA	MEIA	MEIA	
23	[5]	Granada (ES)	218; 82, 136	65–90[c]	148	35	0.300	13	15.9	≤396.4	
					MEIA	RIA	GC-MS	FPIA	MEIA	MEIA	
24	[50]	Boston (US)	1458	70±0.32[g]	148		0.210				131 (m)
					RIA		GC-MS				115 (f)
25	[30]	Santiago de Chile (CL)	491	65–67.9[c]	148				7		
					RIA				RIA		
26	[51]	ND (US)	255	28–82[c]	148		0.376	12.2			
					CLIA		GC-MS	FPIA			
27	[52]	Ontario (CA)	75; 28, 47	80.7±1.5[g]	148			13.3		370	
					CLIA			FPIA		CLIA	
28	[19]	New York (US)	548; 200, 348	77.5 (67–96)[h]	148		0.376	21.3	5.9		124 (m)
					RIA		GC-MS	GC-MS	RIA		106 (f)
29	[12]	Unites States (US)	7233; 3689, 3544	≥3	148		0.370	9	6.8		
					RIA		GC-MS	FPIA	RIA		
30	[53]	Adelaide (AU)	64; 64, 0	50–70[c]	150			10	6.8	317	
					RIA			HPLC	RIA	RIA	
31	[54]	Lieto (FI)	224; 92,132	≥65	150	37	0.450	19.0			
					CPB	RIA	GC-MS	FPIA			
32	[4]	Lausanne (CH)	50; 0, 50	18–91[c]	150		0.260		7		97
					RIA		GC-MS		RIA		
33	[21]	Lund (SE)	209; 91, 118	73±11[g]	150	40	0.410	19.9	7		120
					TR-FIA	RIA	GC-MS	HPLC	RIA		
34	[55]	Puna (IN)	204; 169, 35	27–55[c]	150	35	0.260	15	5		110
					RIA	RIA	GC-MS	HPLC	RIA		
35	[56]	Boston (US)	70; 70, 0	54–81[c]	150			18.5	6.8		
					RIA			HPLC	RIA		
36	[57]	Amsterdam (NL)	1; 1, 0	51	150			18	6.5		
					MEIA			FPIA	MEIA		
37	[58]	Busan (KR)	184; 94, 90	61.6 (22–83)[h]	150	35		12	6.8		
					MEIA	ND		FPIA	ND		
38	[59]	Leiden (NL)	423	≥85	150			13.5	7		
					DCSP			FPIA	DCSP		
39	[10]	Oxford (GB)	1562; 618, 884	≥65	150		0.350	15	5		100
					CPB		GC-MS	FPIA	MBA		

(**Table 1** Continued)

No.	References	City (country)[a]	n (all; m, f)	Age, year	B_{12}[b], pmol/L	HoloTC[b], pmol/L	MMA[b], µmol/L	Hcy[b], µmol/L	sFolate[b], nmol/L	RBC folate[b], nmol/L	Creatinine[b], µmol/L
40	[29]	Great Britain (GB)	1549	≥65	150			20	7		
					CPB			FPIA	MBA		
41	[3]	Lieto (FI)	1048	65–100[c]	150	37		≥15			
					CPB	RIA		FPIA			
42	[60]	Nijmegen (NL)	105; 46, 59	76 (74–80)[h]	150		0.320	19.9			90 (m)
					RIA		GC-MS	HPLC			110 (f)
43	[28]	Araihazar (BD)	1650; 677, 973	20–65[c]	151			11.4 (m) 10.4 (f)	9		
					RIA			HPLC	RIA		
44	[61]	Ibadan (NG)	139; 61, 78	60–98[c]	151						
					HPBC						
45	[62]	India (IN)	36	50.6 (16–80)[h]	155.7						
					CLIA						
46	[6]	Saarland (DE)	545; 251, 294	57 (18–92)[f]	156	35	0.271	12	7		
					CLIA	RIA	GC-MS	GC-MS	CLIA		
47	[63]	Santiago (CL)	108; 41, 67	74.4±3.7[g]	165			14	6.8		
					IAC			FPIA	IAC		
48	[64]	Oslo (NO)	224	18–90[c]	170		0.376	15.0	5		125 (m)
					RIA		CE	HPLC	RIA		115 (f)
49	[65]	Uganda (UG)	280	>18	177						
					ND						
50	[66]	Leiden (NL)	185; 88, 97	≥65	184					124	
		Glasgow (GB)			CLIA					CLIA	
51	[67]	Homburg (DE)	228; 72, 156	>65	196	29	0.280	14.1	11.1		106.1 (m)
					CLIA	RIA	GC-MS	GC-MS	CLIA		79.6 (f)
52	[68]	Aarhus (DK)	937; 349, 588	72 (19–102)[h]	200	40	0.280	11.9		350	133 (m)
					CPB	RIA	GC-MS	FPIA		CPB	120 (f)
53	[23]	Aarhus (DK)	143; 51, 92	72 (24–90)[h]	200	50	0.290	5		50	133 (m)
					CPB	CPB	GC-MS	FPIA		CPB	115 (f)
54	[69]	Gothenburg (SE)	101; 35, 66	52 (18–80)[h]	200	35	0.400	13			
					CPB	ND	GC-MS	HPLC			
55	[70]	Haukeland (NO)	90; 69, 21	38–80[c]	200	40	0.280				
					EIA	ELISA	ND				
56	[71]	Oxford (GB)	116; 43, 73	73[e]	200	40	0.300	14	15		115
					ND	RIA	GC-MS	ND	ND		
57	[72]	Oxford (GB)	2403;986, 1417	≥65	200		0.45				124 (m)
					CLIA		GC-MS				97 (f)
58	[13]	Norway (NO)	6946; 3075, 3871	(47–49 adults)[c]	150;200;400		0.280 adults				106 (m)
				(71–74 elderly)[c]	MBA		0.360 elderly				87 (f)
							GC-MS				

(**Table 1** Continued)

No.	References	City (country)[a]	n (all; m, f)	Age, year	B_{12}[b], pmol/L	HoloTC[b], pmol/L	MMA[b], µmol/L	Hcy[b], µmol/L	sFolate[b], nmol/L	RBC folate[b], nmol/L	Creatinine[b], µmol/L
59	[73]	Albuquerque (US)	100; 50, 50	68–96[c]	221		0.271	16.2	5		
					ND		ND	ND	ND		
60	[26]	Denver (US)	152	65–99[c]	221		0.376	21.6			
					RA		GC-MS	GC-MS			
61	[20]	Seattle (US)	315; 203, 112	65–100[c]	221		0.271	13.9	7.3		109
					ND		ND	ND	ND		
62	[74]	Mashhad (IR)	244; 126, 154	≥65	243.5			15	14.7		
					RIA			ELISA	RIA		
63	[75]	Georgia (US)	103; 21, 82	76±8[g] (60–95)[c]	258		0.271	13.9	6.8	295	127
					RIA		GC-MS	GC-MS	RIA	RIA	
64	[76]	(US) and (ES)	1350; 656, 694	65–90	258			15	14		
					ND			CLIA	ND		
65	[77]	London (GB)	421; 215, 206	66 (37–90)[f]	258		0.271	14			
					EIA		GC-MS	HPLc			
66	[78]	Baltimore (US)	762; 0, 762	≥65	258		0.271	13.9	11.4		100
					CPB		GC-MS	GC-MS	CPB		
67	[2]	Hanover (DE)	178; 0, 178	60–70[c]	258		0.271	12	7	320	
					CLIA		GC-MS	FPIA	CLIA	CLIA	
68	[79]	Chicago (US)	121	≥65	258		0.271				
					CDI		GC-MS				
69	[18]	Molise (IT)	240; 161, 79	18–66	350			10	15	305	
					ILA			HPLC	ILA	ILA	

[a]ISO 3166-1 country code elements; [b]SI conversion factors: To convert vitamin B_{12} (pg/mL) to pmol/L, multiply with 0.738; vitamin B_{12} and HoloTC to ng/L, multiply with 1.3554; folate to ng/mL, multiply with 2.266; MMA to mg/L, divide by 8.475; Hcy to mg/L, divide by 7.397; creatinine to g/dL, divide by 88.4; [c]range; [d]mean (95% CI); [e]mean; [f]mean (range); [g]mean ± SD; [h]median (range). CDI, competitive displacement immunoassay; CE, capillary electrophoresis; CLIA, chemiluminescent immunometric assay; CPB, competitive protein binding; DCSP, dual count solid phase no boil-assay; EIA, enzyme immunoassays; ELISA, enzyme-linked immunosorbent assay; f, female; FPIA, fluorescence polarization immunoassay; GC-MS, gas chromatography-mass spectroscopy; HPLC, high pressure liquid chromatography; IAC, ion capture assay; ILA, immunoligand assay; LC-MS, liquid chromatography-mass spectroscopy; m, male; MBA, microbiological assay; MEIA; microparticle enzyme immunoassay; ND, there was no date or no specific method; RA, radiodilution assay; RIA, radioimmunoassay; TRFIA, time-resolved fluoroimmunoassay.

Methylmalonic acid

A total of 44 articles included MMA as biomarker to assess vitamin B_{12} deficiency. Reported values ranged from 0.210 to 0.470 μmol/L. The majority of GC-MS users (77.3%) reported very close cut-offs: from 0.26 to 0.28 μmol/L. Only one author disclosed different MMA cut-off for adults and elderly [13].

Homocysteine

Hcy was measured in 53 studies and cut-off values ranged from 10 μmol/L [18] with HPLC to 21.6 μmol/L [26] with GC-MS. Only seven studies did not specify the analytical method used. FPIA was the preferred method (39%) to determine Hcy levels, followed by HPLC (32.6%) and GC-MS (21.7%). Fifteen studies set Hcy cut-off at 15 μmol/L and this value was the most commonly used. Two studies divided samples by gender, reporting 17.1 μmol/L and 16.8 μmol/L [27], and 11.4 μmol/L and 10.4 μmol/L for males and females, respectively [28].

sFolate

This variable was measured in 40 studies and the method mostly used for analysis was RIA (53%). The most common cut-off used was 6.8 nmol/L (22.5% of studies), followed by 7 nmol/L (17.5%), although values ranged from 3.7 nmol/L (RIA) to 15.9 nmol/L (MEIA).

RBC folate

Only 14 articles (20.6%) included RBC folate measurement. All studies used different cut-off concentrations which ranged between 124 nmol/L (CLIA) and 397 nmol/L (MEIA). The most common methodology was CLIA.

Creatinine

Creatinine values were separated by gender in 13 articles, reporting a cut-off range from 90 μmol/L to 141 μmol/L for males and 79.6 μmol/L to 120 μmol/L for females. Fifteen articles did not separate data by gender, giving a cut-off interval from 97 μmol/L to 141 μmol/L. The majority of studies have considered 120 μmol/L or 124 μmol/L as high reference range limit for males and 115 μmol/L for females.

Discussion

A major problem when comparing vitamin B_{12} deficiency prevalence across studies consisted on the variability of cut-off values used. Several factors can be addressed in this context. The first one was the method used to establish cut-offs values since means, medians, two or three SD or percentiles and the analytical methods used to measure biomarkers were diverse. Another issue was the application of values obtained from younger subjects to the elderly. All these factors contributed to scattered cut-off values. This is the first review to group information on cut-off values for vitamin B_{12} deficiency from 1992 to 2014, taking into account age and analytical detection methods.

Regarding the literature, the prevalence of vitamin B_{12} deficiency in British elderly ranged from 5% (age 65–74 years) to 10% (age >75 years) with 150 pmol/L as a cut-off [29]. Sanchez et al. found 12% B_{12} deficiency using 148 pmol/L and 25.4% with cut-off value of <221 pmol/L [30]. Meanwhile, Palacios et al. [5] found 17.4% (≤148 pmol/L) and Loikas et al. [3] observed 6.1% considering 150 pmol/L as cut-off. Two extreme B_{12} cut-offs were recorded: the lowest one (123 pmol/L) was used by Valente et al. and observed 8% of B_{12} deficiency [22]. However, Zapacosta et al. using the highest value (350 pmol/L) found that only 16.3% of the Italian adults presented an adequate vitamin B_{12} concentration [18]. A detailed study on vitamin B_{12} status was carried out by Vogiatzoglou et al.; using 150 pmol/L as cut-off, they found a prevalence of 0.4% for adults and 1.7% for elderly [13].

Only four authors used vitamin B_{12} as a single marker, while the rest of them made a combination of markers, as already proposed by Valente et al. [22] and Palacios et al. [5]. The biomarker most included was Hcy (78%) followed by MMA (65%), sFolate (59%), HoloTC (30%) and RBC folate (21%). The small number of studies including RBC folate was unexpected, as erythrocyte folate is considered a more precise physiological marker of folate concentrations than sFolate. This may be due to the preparation of samples for RBC folate measurement which requires more laborious steps than for sFolate. Additionally, both folate markers should be measured to assess if there is a mixed deficiency between folate and vitamin B_{12} [1]. However, MMA measurement is important to distinguish between vitamin B_{12} and folate deficiency. Elevated MMA is a specific marker of vitamin B_{12} deficiency while Hcy rises in both vitamin B_{12} and folate deficiencies [2, 31].

The majority of studies did not take into account age or gender of participants when they applied the cut-offs values. For example, none of the studies modified the cut-offs for vitamin B_{12} depending on age and only two

for MMA. Vogiatzoglu et al. [13] set different cut-off values for middle-ages (47–49 years; 0.280 μmol/L) and elderly (71–74 years; 0.360 μmol/L) due to different creatinine and vitamin B_{12} concentrations. In the same way, Clarke et al. took in consideration both age and renal function to adapt cut-offs because their study was carried out in elderly [10]. In the elderly, impaired renal function could be an important confounding factor for Hcy and MMA [32]. For this reason, we have included in the Table 1 the creatinine cut-off values when available. In the same way, none of the studies included different cut-offs for Hcy depending on age. Only Carmel et al. [27] and Gamble et al. [28] reported values for males (m) and females (f) separately: 17.1 μmol/L (m) and 16.8 μmol/L (f), and 11.4 μmol/L (m) and 0.4 μmol/L (f). Age and gender did not seem to influence the choice of cut-offs values for both sFolate and RBC folate.

MMA was predominantly measured using GC-MS methods. This method is expensive and not available in all the routine laboratories. Therefore, Palacios et al. proposed an algorithm to differentiate vitamin B_{12} from folate deficiency with no need for MMA measurement [5].

Countries that currently mandate folic acid fortification as well as individuals under multivitamin supplementation may require specific reference values for the diagnosis of vitamin B_{12} deficiency [33]. Vitamin B_{12} supplements or food fortification should be taken into account in countries that use this kind of supplementation. A study by Sanchez et al. showed that a daily dose of 1.4 ug of vitamin B_{12} was not sufficient to improve vitamin B_{12} status [30]. Supplementation with higher doses of vitamin B_{12} (>500 ug/day) have proven effective in Latino populations residing in USA [34] and institutionalized Spanish elderly increased significantly from 308.4 pmol/L to 558.3 pmol/L ($p<0.001$) [35]. Also, Favrat et al. showed that oral vitamin B_{12} treatment normalized the metabolic markers [4]. However, this response did not persist for an additional 3 months following cessation of therapy. These discrepancies may be the result of employing the same cut-offs for diagnosing vitamin B_{12} deficiency in populations exposed to different basal levels of vitamin B_{12} and folate. Vitamin B_{12} is not the only vitamin affected since folate is also added to food in some countries. In these countries with food folate fortification or populations taking folic acid supplements, the upper reference limit for Hcy is usually 20%–25% lower than in non-fortified populations [36]. Clarke et al. suggested as ultimate gold standard for vitamin B_{12} deficiency the reduction in increased Hcy or MMA concentrations and improvement in clinical symptoms or signs in response to vitamin B_{12} treatment [10].

Limitations of this review comprise the lack of information on preanalytical procedures, analytical performance and diagnostic accuracy, even if these factors may have a deep impact on cut-off values choice. Unfortunately, the majority of articles did not specify these kinds of data. Therefore, we were not able to include them in our review.

Conclusions

There is no consensus in the literature about cut-off points for blood vitamin B_{12} reference ranges and its associated metabolites. In most of the studies, age and gender were not taken into account when measuring any of these biomarkers, nor the use or consumption of fortified food. Published data in the literature regarding deficiency percentage should be considered with caution. For future studies on vitamin B_{12} status, it would be necessary to establish different reference cut-offs according to age and gender, considering the analytical methods used.

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