Review

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Serum α-fetoprotein in pediatric oncology: not a children's tale

https://doi.org/10.1515/cclm-2018-0803
Received July 27, 2018; accepted September 11, 2018

Abstract

Background: Measurement of α-fetoprotein (AFP) concentrations in the serum of infants is useful for the management of testicular germ cell tumors, hepatoblastoma and hepatocellular carcinoma. Here, we provide a critical review of the available information about pediatric reference intervals (RI), focusing on their utility in interpreting AFP as an aid for cancer diagnosis.

Content: Evidence sources in the available literature were critically appraised. Out of 3873 retrieved papers, 24 were finally selected and carefully inspected, and six of them overcame exclusion criteria (i.e. methodological limitations in the study design, statistical gaps, drawbacks in traceability of the AFP assay to higher order materials and/or biased reporting of AFP results). Preterm and term infants up to the 3rd month of life exhibited the highest average AFP concentrations, but the attempt of defining RI by data pooling and partitioning for age intervals was impeded by the wide variability of data. The inability of defining robust RI in the first months of life made difficult, if not impossible, using upper reference limits for ruling out malignancies with a single AFP result. Evaluating the behavior of AFP concentrations 5 days from the baseline result, if this exceeds risk thresholds partitioned for age, according to the formula $X_t = X_0 * 2^{-t/HL}$ (where: t = days elapsed for AFP retest; HL = AFP half-life according to age; X_0 = AFP baseline concentration, and X_t = predicted AFP concentration at day 5), could give a better information.

Summary: Novel studies defining AFP RI in infants based on robust methodology are warranted to improve the interpretation of AFP results in pediatric oncology. In the meantime, algorithms based on both serum AFP absolute concentrations and HL may aid in cancer diagnosis.

Keywords: germ cell tumors; hepatoblastoma; hepatocellular carcinoma; pediatrics; reference interval; α-fetoprotein.

Introduction

α-Fetoprotein (AFP) is a 70-kDa glycoprotein, structurally similar to albumin and mostly synthesized during embryonic development by the fetal yolk sac and liver. Its determination in serum in pediatrics has been recommended for diagnosis, monitoring and treatment of germ cell tumors (GCT), hepatoblastoma (HB) and hepatocellular carcinoma (HCC) [1–9]. However, uniform approaches for AFP interpretation are lacking. Firstly, recommendations have been mostly developed on dated studies using not standardized AFP assays [10] and this may likely affect the generalization of the derived clinical decision making to the present day. Secondly, serum AFP concentrations are physiologically high in newborns because of fetal production, and clinicians may often be doubtful about the interpretation of an AFP result in an infant. Looking at AFP concentrations alone could not reliably exclude tumors in infants, and often, histologic detection and imaging should be prioritized [5, 8, 9]. In general, there is an agreement about the low capability of AFP to rule out malignancy in preterm and term infants up to 4 months of age, as available upper reference limits (URL) look unreliable and unsuitable for clinical application [1, 11]. Practical indications about the AFP use in young infants are therefore retrieved from literature based on registries/retrospective case series collected in years of clinical experience [12–20].

Given the mentioned issues, it seemed appropriate systematically appraising the available information about reference intervals (RI) of AFP in serum of infants, focusing on factors influencing data and on potential bias due to methodological limitations, in the function of the role of marker in pediatric oncology.

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Estimation of AFP RI in the pediatric population: evidence and pitfalls

Data sources and searches

The peer-reviewed literature published since 1965 up to May 30th, 2018 was searched using Medline (PubMed) and Embase databases, with "alpha fetoprotein", "reference range/value" and "paediatric" as MeSH Terms and Title, setting as filters: humans, English language, newborn/child age from birth to 18 years. The research resulted in 3873 papers. Among those, 19 papers reporting in the abstract text the estimation of AFP RI in newborn/infant/pediatric populations were selected. Five additional publications were retrieved from the references of those papers. A total number of 24 papers was therefore identified: 22 experimental studies [10, 11, 21–40] (one including two substudies [38]) and two abstracts from poster presentations [41, 42]. The main features of the selected studies are reported in Table 1.

Effect of sex, gestational age and birth weight on serum AFP

One third of studies reported RI partitioned for gestational age (GA) (preterm vs. term newborns) using 36 weeks as the threshold [21–23, 25, 27, 28, 30, 32]. Others considered a miscellaneous of preterm and term [11, 24, 26, 34] or only term (and near term) newborns [10, 29, 31, 35, 36, 41]. Importantly, all studies performed in the last 20 years using assays on automated platforms (except for that by Coakley et al. [11]) did not report information about GA, providing only cumulative RI. Prematurity is widely recognized as a cause for raised AFP concentrations in serum, due to the liver and gut growth [21], so that knowledge of GA is required for adjusting AFP concentrations in infants up to ~1 year of age. At birth, term babies may have AFP concentrations 8–10-fold lower than preterm newborns (at 27th–35th week of GA) [30, 32]. Some authors have proposed to report AFP RI adjusted for GA deficit [11, 23].

AFP appears to increase in low-birth weight newborns independently of sex [24, 28, 29, 34, 36]. Accordingly, some authors proposed further adjusting AFP RI for birth weight, hypothesizing that AFP synthesis in low-birth weight infants may continue after birth [28, 36]. There is some evidence that (independently of weight) the AFP synthesis does not cease entirely at birth, because hepatocytes are still producing the protein up to the first month of life [10]. This hypothesis fits with the semi-logarithmic shape of the AFP pattern in early postnatal life and with the non-linear increasing half-life (HL) describing the rate of serum AFP disappearance [10, 27]. Both in preterm and term infants, it has been described as a biphasic pattern on a semi-logarithmic plot consisting of a first rapid rate of AFP reduction followed by a slower phase. The one difference was that in preterm babies the rapid decline lasted for 4 months, reducing to 2 months in term infants. At the end of this GA-dependent period of rapid decrease, AFP values decrease far more slowly, without significant dependence from GA [32].

Some authors suggested sex-partitioned RI at birth [26, 29, 30, 41] and up to the 1st week of life [26], from 6 months to 6 years [38], or from birth to 18 years [41, 42]. Noteworthy, the evidence widely varied across different studies and pediatric ages, and most of studies did not agree with sex partitioning either in the first days/weeks of life or in older ages.

One study showed that newborns from smoking mothers have higher AFP concentrations at birth [35].

RI partitioning for pediatric ages

Studies estimated RI at various pediatric ages and intervals of age. We were able to identify a total number of 70 types of age partitions (e.g. birth, day 1, 7–14 days, month 1, 6–12 months, year 1, 1–2 years, etc.) (Table 1). Studies provided RI:

(a) only at birth [25, 29, 30, 34, 35];
(b) day by day from birth/day 1 to the first days or to 1–2 weeks of life [22, 24, 26, 28, 36];
(c) from birth (in the first months every 1–2 weeks and thereafter 1–2 months) up to when AFP concentrations were comparable to those of adult population (i.e. from 8 to 36 months, according to different studies) [21, 27, 31, 32];
(d) for wide age intervals (e.g. 0–6 months, 1–3 years) [33, 37, 41, 42];
(e) by graphical analysis with prediction bands supported by regression equations theoretically enabling to estimate AFP values at any age, often adjusted for other factors, such as GA or birth weight [10, 33, 34].

Considering the AFP behavior in the early postnatal life described above, RI directly estimated by experimental data (and not retrieved by equations) and partitioned for narrow age intervals from birth/first days of life up to the 1st year of age are undoubtedly more reliable than those collapsing several age periods. The simple visual inspection of individual data, unfortunately often disregarded,

Table 1: Main features of retrieved studies.

Authors (year)	Setting	GA[a,b]	Infant age	Sample size[c]	AFP factors of variation[d]	AFP RI descriptive statistics	Sample type (matrix)	Method/Assay	Traceability	AFP half-life
Karlsson et al. (1972) [24]	Pediatrics department	Preterm + term	Birth, 1 day, 14 days	114	Sex *GA* *Weight*	Mean	Umbilical cord blood at birth; venous blood (serum)	IE	Internal standard	ND
Hyvarinen et al. (1973) [25]	Pediatrics department	Preterm/term	Birth	22 preterm 105 term	*GA*	Mean ± 2SD	Umbilical cord blood at birth; venous blood (serum)	RID	Internal standard	Term at birth: 7 days
Caballero et al. (1977) [26]	Obstetrics ward	Preterm + term	Birth, 3, 5, 6 days	6 preterm 42 term	*Sex* *GA* *Weight*	Mean	Umbilical cord blood at birth; venous blood (serum)	RIA	Internal standard	At birth: 5–6 days
Tsuchida et al. (1978) [10]	Pediatric surgery/ Obstetrics ward	Term	From birth up to 14 years	10 newborns 45 infants aged 26 days–14 years	ND	Prediction bands (graph) and equation	Venous blood (serum)	RIA	Internal standard	ND
Wu et al. (1981) [27]	Pediatrics department	Preterm/term	Term: birth-2 weeks, 2–4 weeks, 1, 2, 3, 4, 5, 6, 7, 8 months Preterm: 7–14 days	10 preterm 148 term (32 infants <4 months and 116 infants <1 year)	ND	Mean ± 2SD	Capillary blood (serum)	RIA	WHO 72/227	Term birth-2 weeks: 5.5 days Term 2 weeks–2 months: 11 days Term 2–4 months: 33 days
Mizejewski et al. (1983) [28]	Pediatrics department	Preterm/term	1, 2, 3, 4, 5, 6, 7 days	83 preterm 479 term	Sex *GA* *Weight*	Mean ± 2SD	Heel-prick blood (dried blood)	RIA	WHO 72/225 WHO 72/227	Term: 5.7 days Preterm: 7 days
Obiekwe et al. (1985) [29]	Obstetrics ward	Term	Birth	105	Sex *GA* *Weight*	Mean	Umbilical cord blood at birth	RIA	WHO 72/225	ND
Goraya et al. (1985) [22]	Special care baby unit	Preterm/term	1, 2–7, 8–14, 15–28, 29–35 days	55 preterm 6 term	*GA* *Weight*	Median (min–max)	Umbilical cord blood at birth; venous blood (plasma)	RIA	WHO 72/225	ND
Blair et al. (1987) [23]	Pediatric surgery	Preterm/term	Birth, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 weeks	37 preterm 19 term	Sex *GA*	Mean ± 2SD	Capillary blood; venous blood (plasma)	RIA	WHO 72/225	ND
Bansal et al. (1989) [30]	Obstetrics ward	Preterm/term	Birth	13 preterm 39 term	*Sex* *Weight*	Mean ± 2SD	Umbilical cord blood	In-house ELISA	Internal standard	ND

Table 1 (continued)

Authors (year)	Setting	GA[a,b]	Infant age	Sample size[c]	AFP factors of variation[d]	AFP RI descriptive statistics	Sample type (matrix)	Method/Assay	Traceability	AFP half-life
Lee et al. (1989) [31]	Obstetrics ward/ Pediatrics department	Term	Birth, 1–15, 16–30 days, 1, 2, 3, 4–5, 6–7, 8–9, 10–12 months	284	*Sex*	Mean ± 2SD	Blood (NA)	RIA (Dainabot Radioisotope Laboratory)	NA	ND
Lahdenne et al. (1991) [32]	Obstetrics ward	Preterm/term	Preterm and Term: Birth, 2, 4, 6, 9, 12 months Preterm: 1, 2, 4, 6, 10, 12 weeks	15 preterm (53.3% M) 12 term (33.3% M)	*GA*	10th–90th percentile	Blood (serum)	RIA	WHO 72/225	ND
Soldin et al. (1992) [41]	Hospital-based pediatric population	Term	Birth-1 month, 1–12 months, 1–3 years, 4–6 years, 7–12 years, 13–18 years	344 (53.5% M): age 0–1 year 250 (53.6% M): age 1–3 years 477 (50.1% M): age 4–12 years 267 (54.3% M): age 13–18 years	*Sex*	2.5th–97.5th percentile	Blood (NA)	EIA (Abbott Diagnostics)	WHO 72/225	ND
Ohama et al. (1997) [33]	Pediatric surgery/ obstetsrics ward	NA	0–2 years	163	ND	Prediction bands (graph) and equation	Blood (NA)	RIA (Dainabot Radioisotope Laboratory)	NA	Day 10: 3 days Day 20: 7 days Day 30: 10 days Day 40: 14 days Day 50: 17 days Day 60: 21 days Day 70: 24 days Day 80: 28 days Day 90: 31 days
Blohm et al. (1998) [21]	Obstetrics ward/ Pediatrics department	Preterm/term	0, 1, 2, 3, 4, 5, 6, 7, 8–14, 15–21, 22–28, 29–45, 46–60 days, >2–3, >3–4, >4–5, >5–6, >6–24 months	90 preterm 256 term	Sex *GA* Weight *Hyperbilirubinemia*	95.5% central range	Capillary blood; venous blood (serum)	EIA (Abbott Diagnostics)	WHO 72/225	Term 0–28 days: 5.1 days Term 29–45 days: 14 days Term 61–90 days: 28 days Term 121–150 days: 42 days

Table 1 (continued)

Authors (year)	Setting	GA[a,b]	Infant age	Sample size[c]	AFP factors of variation[d]	AFP RI descriptive statistics	Sample type (matrix)	Method/Assay	Traceability	AFP half-life
										Preterm 0–28 days: 6 days Preterm 29–60 days: 14 days Preterm 61–180 days: 28 days Preterm 181–720 days: 100 days
Bellini et al. (1998) [34]	Neonatal intensive care unit	Preterm+term	0–1 day	150 (47% M)	Sex *GA* *Weight*	Mean ± 2SD; graph and prediction band	Capillary blood (serum)	RIA (Serono)	WHO 72/225	ND
Beratis et al. (1999) [35]	Obstetrics ward	Term	Birth	103	*Smoking mother*	Mean ± 2SD	Umbilical cord blood (serum)	EIA (Abbott Diagnostics)	WHO 72/225	ND
Dugaw et al. (2001) [42]	Hospital-based pediatric population	NA	0–30 days, 1–3 months, 4 months–18 years	66	ND	Mean ± 2SD	Blood (NA)	CMIA (Vitros ECi Ortho Clinical Diagnostics)	WHO 72/225	ND
Bader et al. (2004) [36]	Neonatology	Term	60 ± 24 h	260	Sex Ethnicity *GA* *Weight*	95% central range	Umbilical cord blood at birth; venous blood (serum)	RIA DELFIA (Wallac)	WHO 72/225	ND
Coakley et al. (2005) [11]	Hospital-based pediatric population	Preterm+term	Birth–110 days	94	*GA*	5th–95th percentile	Venous blood (plasma)	CMIA (AxSYM Abbott Diagnostics)	WHO 72/225	ND
Soldin et al. (2008) [37]	Hospital-based pediatric population	NA	0–3, 3–6, 6–12 months, 1–3, 3–18 years	557	*Sex*	2.5th–97.5th percentile	Venous blood (serum)	CMIA (Immulite 2000 Siemens)	WHO 72/225	ND
La'ulu et al. (2011) [38]	Pediatric Surgery	NA	6–11 months 1, 2, 3–6 years	913	*Sex*	95% central range	Venous blood (serum)	EIA (Access Beckman Coulter)	WHO 72/225	ND
				886				ECLIA (Modular E170 Roche Diagnostics)	WHO 72/225	

Table 1 (continued)

Authors (year)	Setting	GA[a,b]	Infant age	Sample size[c]	AFP factors of variation[d]	AFP RI descriptive statistics	Sample type (matrix)	Method/Assay	Traceability	AFP half-life
Bailey et al. (2013) [39]	Hospital-based pediatric population	NA	3–6, 6–12 months 1–3, 3–18 years	1145	Sex	2.5th–97.5th percentile	Venous blood (serum)	CMIA (Architect i2000 Abbott Diagnostics)	WHO 72/225	ND
Bevilacqua et al. (2014) [40]	Hospital-based pediatric population	NA	0–1, 1–6, 6–12 months 1–19 years	466	Sex	2.5th–97.5th percentile	Venous blood (serum)	CMIA (Architect ci4100 Abbott Diagnostics)	WHO 72/225	ND

[a]GA, gestational age; AFP, α-fetoprotein; RI, reference interval; IE, immunoelectrophoretic method; ND, not determined; RID, radial immunodiffusion; NA, not available; EIA, enzyme immunoassay; CMIA, chemiluminescent immunoassay; DELFIA, dissociation enhanced lanthanide fluorescence immunoassay; ECLIA, elettrochemiluminescent immunoassay. [b]Preterm/term: results not partitioned for GA; Preterm/term: results partitioned for GA. [c]Refers to the actual number of tested subjects. The absolute number of preterm and term babies and percentage of males (M) is reported, when available. [d]Factors responsible of significant variations of AFP concentrations in serum are reported in italics.

should enable to create partitions in different studies, similar in terms of their central locations and spread of individual values, as well as practical for clinical use [43]. This is relevant for the considerable incidence of tumors requiring AFP determination in the first months of life. For instance, sacrococcygeal GCTs occur with a consistent proportion of infants younger than 7 months [12, 20]. Sixty-nine percent of yolk sac tumors (YST) are in neonates and, when presenting in the fetal period (<30 weeks of GA) often occur with teratomas (one out of 40,000 live births), characterized by poor prognosis and high mortality rate [5, 12, 15].

Another issue concerns the identification of the infant age exhibiting AFP values similar to concentrations reported in the adult population. Depending on an assay used, physiological AFP concentrations in adults vary between 5 and 10 μg/L [37, 38, 41]. It is odd to note the wide variability of proposed ages to reach these values: after 8 months for Wu et al. [27], after the 2nd year of life for Blohm et al. [21], or after the 3rd year for other authors [37–39, 41].

The importance of the AFP assay

Studies on AFP RI, quoted by running international protocols [44, 45], were performed 35–40 years ago using RIA methods calibrated against internal standards. As previously discussed for other important biomarkers [46], the lack of traceability of those methods to common higher order reference materials represents a great limitation to the use of derived RI when AFP is measured by differently calibrated assays. Harmonization for assays should therefore be pursued as method-related differences may significantly affect clinical decision-making. At present, the harmonization of AFP assays still relies on the implementation of traceability to the WHO international standard coded 72/225, established in 1975, value assigned in international units (IU) and consisting of a relatively crude freeze-dried preparation of 50% v/v cord serum in phosphate buffer containing albumin. The relationship between μg and IU usually is given as 1.21 μg = 1000 IU, but conversion factors may widely vary by assay and this may represent a significant source of inter-assay variability and bias. Furthermore, the commutability of WHO 72/225 preparation has been questioned [47] and some authors have observed that running immunoassays still suffer from significant inter-method variability limiting the comparability of AFP results [48].

To judge the harmonization status of AFP measurements, it sounds relevant to refer to external quality assessment programs. In the 1980s, the inter-assay

variability of AFP assays was around 20% for concentrations ≥20,000 IU/L [49]. Over the following 30 years, the inter-assay CVs have markedly improved and UK NEQAS surveys have recently indicated an average CV of 5.2% (calculated for all specimens issued in 2015), representing the lowest inter-assay variability in the field of tumor markers [50]. For sure, the plain molecular structure of AFP may help in decreasing differences in antibody selectivity of different immunoassays. On the other hand, imprecision of assays frequently appears to be yet an issue for their clinical use [48].

Statistical drawbacks

Across different studies, AFP concentrations and RI have been variably summarized, using geometric mean, mean ± 2SD, median, 2.5th–97.5th or 5th–95th percentile intervals (Table 1), usually without checking or, by default, assuming normal distribution of values. Alternatively, some papers provided AFP RI by graphs with prediction bands according to regression equations, theoretically enabling to estimate RI at fixed ages.

The main limitation to all studies was the very low size of samples collected at early ages and for each partition. Tsuchida et al. [10] estimated RI by assaying AFP concentrations in 10 newborns at birth and 45 infants aged 26 days–14 years. Other authors accounted for ≤5 infants for each age interval [21, 27], while some studies did not report the sample size at all [42]. The poor sample size must be emphasized since these studies are commonly quoted by routinely adopted clinical protocols [1, 7, 44, 45, 51]. Erroneous RI may be even obtained whether outliers were not checked and removed.

Summarizing evidence

From available data, we are unaware about the actual burden of variability contributed by the between-subject biological variation (especially within the 1st year of age) and that attributable to the inaccuracy of estimates for the already described gaps. After excluding most of retrieved studies according to various methodological drawbacks (Table 2), we summarized in Table 3 data from the remaining articles [21, 22, 32, 35, 36, 38], which provide some evidence about RI for age partitions of clinical relevance in pediatric oncology. It should be noted that we also excluded the two studies reporting AFP concentrations in IU [11, 29] for the impossibility of comparing data with other studies overcoming exclusion criteria, even if they did not show other specific problems.

From data in Table 3, we may observe that:

(f) the pattern of AFP release in preterm and term infants should imply considering only narrow age RI in the first 4 months of age and, possibly, day-by-day RI in the first 2 weeks of life;
(g) the variability of RI and URL is striking for early age partitions at least up to 6 months of age;
(h) more than 8 months of age are required because AFP concentrations in term babies decrease to values of adult population. In preterm infants, this possibly requires more than 1 year of life.

Overall, the wide variability of RI prevents the estimation of pooled URL partitioned for ages, which may enter in the laboratory report. Furthermore, no study is completely free of methodological limitations to provide stronger evidence than others are.

AFP in pediatric oncology

HB and HCC

AFP determination and monitoring are recommended for screening high-risk pediatric populations for early rule out of HCC and HB, the former prevalent at >3 years and the latter in intrauterine life and between 6 months and 3 years

Table 2: Exclusion criteria of retrieved studies according to methodological drawbacks.

Study references	Drawbacks
[24, 26, 34]	Providing reference intervals (RI) estimated by mixing preterm and term infants
[28]	Using different matrix (not serum/plasma) for assaying AFP concentrations
[10, 24–27, 30, 31, 33]	Using AFP assays not traced to WHO 72/225 reference material or with unavailable data on traceability
[10, 33]	Reporting regression equations and/or graph with prediction bands enabling only rough/visual comparisons
[28]	Reporting AFP concentrations partitioned only for specific categories (e.g. weight class)
[25, 42]	Lacking data on the sample size for age partitions
[37, 39–41]	Reporting RI for a too wide age interval (i.e. birth–1 month, 3–6 months) in the 1st year of age

Table 3: Reference intervals for preterm and term infants in the selected studies.

Age	Reference	Sample size	Assay	Statistics	Reference interval, µg/L
Preterm infants					
Birth	[21]	4	EIA Abbott	95.5% central range	31,300–799,800
	[32]	8	RIA home made	10th–90th percentile	149,700–335,375
1 day	[21]	4	EIA Abbott	95.5% central range	27,800–711,200
	[22]	19	RIA Amersham Intl.	Min–max range	15,300–333,600
2 days	[21]	4	EIA Abbott	95.5% central range	24,700–632,400
3 days	[21]	4	EIA Abbott	95.5% central range	22,000–562,300
4 days	[21]	4	EIA Abbott	95.5% central range	19,500–500,000
5 days	[21]	4	EIA Abbott	95.5% central range	17,400–444,600
6 days	[21]	4	EIA Abbott	95.5% central range	15,400–392,700
1 week	[21]	4	EIA Abbott	95.5% central range	12,600–350,000
	[22]	32	RIA Amersham Intl.	Min–max range	3300–290,400
	[32]	10	RIA home made	10th–90th percentile	43,825–234,500
2 weeks	[21]	4	EIA Abbott	95.5% central range	6000–312,000
	[22]	18	RIA Amersham Intl.	Min–max range	15,300–154,200
3 weeks	[21]	4	EIA Abbott	95.5% central range	2700–151,400
2 weeks–1 month	[22]	16	RIA Amersham Intl.	Min–max range	9500–70,200
1 month	[21]	4	EIA Abbott	95.5% central range	1200–118,900
	[32]	14	RIA home made	10th–90th percentile	21,825–66,460
1 month–6 weeks	[21]	5	EIA Abbott	95.5% central range	389–79,400
6 weeks	[22]	6	RIA Amersham Intl.	Min–max range	4600–86,900
	[32]	10	RIA home made	10th–90th percentile	16,250–47,435
2 months	[32]	7	RIA home made	10th–90th percentile	3100–36,900
3 months	[32]	11	RIA home made	10th–90th percentile	560–10,140
4 months	[32]	14	RIA home made	10th–90th percentile	75–621
6 months	[32]	15	RIA home made	10th–90th percentile	22–170
9 months	[32]	14	RIA home made	10th–90th percentile	6–54
1 year	[32]	14	RIA home made	10th–90th percentile	5–33
15 months	[32]	13	RIA home made	10th–90th percentile	2–28
Term infants					
Birth	[21]	13	EIA Abbott	95.5% central range	9000–191,000
	[32]	8	RIA home made	10th–90th percentile	30,875–44,350
	[35]	103	EIA Abbott	Mean ± 2SD	<0.4–132,000
	[36]	184	RIA DELFIA	95% central range	15,000–147,000
1 day	[21]	13	EIA Abbott	95.5% central range	7900–166,000
	[22]	8	RIA Amersham Intl.	Min–max range	3800–151,500
2 days	[21]	13	EIA Abbott	95.5% central range	7000–144,500
3 days	[21]	13	EIA Abbott	95.5% central range	6000–126,000
	[36]	241	RIA DELFIA	95% central range	9.7–111,900
4 days	[21]	13	EIA Abbott	95.5% central range	5300–109,700
5 days	[21]	13	EIA Abbott	95.5% central range	4600–96,600
6 days	[21]	13	EIA Abbott	95.5% central range	4100–84,300
7 days	[21]	13	EIA Abbott	95.5% central range	3500–73,600
7–14 days	[21]	13	EIA Abbott	95.5% central range	1500–59,000
	[22]	3	RIA Amersham Intl.	Min–max range	6300–24,800
15–21 days	[21]	13	EIA Abbott	95.5% central range	575–22,900
22–28 days	[21]	13	EIA Abbott	95.5% central range	316–6300
1 month–6 weeks	[21]	10	EIA Abbott	95.5% central range	30–5800
6 weeks–2 months	[21]	10	EIA Abbott	95.5% central range	16–2000
2 months	[32]	12	RIA home made	10th–90th percentile	88–412
4 months	[32]	11	RIA home made	10th–90th percentile	16–127
6 months	[32]	10	RIA home made	10th–90th percentile	11–67
>8 months	[32]	11	RIA home made	10th–90th percentile	5–27
1 year	[32]	10	RIA home made	10th–90th percentile	4–17
	[38]	72 F	EIA Access Beckman	95% central range	0.2–41
	[38]	65 M	EIA Access Beckman	95% central range	1–17

Table 3 (continued)

Age	Reference	Sample size	Assay	Statistics	Reference interval, µg/L
	[38]	67 F	ECLIA E170 Roche	95% central range	1.8–38
	[38]	64 M	ECLIA E170 Roche	95% central range	1.4–21
2 years	[38]	134	EIA Access Beckman	95% central range	0.6–12
	[38]	131	ECLIA E170 Roche	95% central range	1.2–15

of age [52]. However, the diagnostic accuracy of AFP is far limited, especially in the first year of life, for the difficult interpretation of its results [8, 9, 52, 53]. Data about the diagnostic sensitivity of AFP for the different histologic variants of HCC are also sparse, even if in case of fibrolamellar HCC AFP concentrations were almost always unchanged [54, 55]. The AFP capability to discriminate HB from hemangioendothelioma, prevalent at the same ages of HB, has been questioned, particularly in fetuses and neonates, whereas in older children it is increased [9]. AFP concentrations exceed the URL only in 50% of patients with HB, because the AFP of tumor origin is often not enough to contribute significant rising of baseline values in early neonatal life.

AFP values may also have prognostic value: marker concentrations >100 µg/L generally characterize standard-risk HB, whereas very high-risk HB show very low (<100 µg/L) or extremely high AFP concentrations (≥1,000,000 µg/L) [8]. After weeks from surgery, if no residual tumor is left, AFP is expected to decrease under the corresponding URL. Minimal AFP increases are associated with liver regeneration, whereas persistently high serum AFP concentrations and/or slower decrease may indicate the persistence of active disease or relapse [7]. The low rate of decrease of AFP during chemotherapy predicts adverse outcome and AFP normalization according to RI for age is regarded as one criterion to assess complete response to treatment [56]. Progression of the disease accounts for an unequivocal increase of serum AFP concentrations (detected by serial determinations every 1–2 weeks), even without clinical (physical and/or radiological) evidence of tumor re-growth [7]. In children with unresectable or metastatic HB, a decline of <2 logs in AFP concentrations may identify poor responders to therapy that should be considered for alternative treatment [57].

GCT

In GCT, serum AFP and β-human chorionic gonadotropin (hCGβ) measurements, imaging and histological examination, together with tumor location (brain, sacrococcygeal, mediastinum, gonadal), are required for diagnosis [1]. AFP increases may be variably associated to hCGβ. In infants and adolescents, who do not have underlying liver disease, increases of AFP or hCGβ values indicate significant secreting components of YST or choriocarcinoma, respectively, and may rule out pure mature teratomas or seminomas, though the latter may secrete minimal amounts of hCGβ [2, 13]. AFP secretion characterizes malignant GCT with YST differentiation, which should however be confirmed by immunohistochemistry [4]. The histologic detection of foci of YST (e.g. in sacrococcygeal teratomas) is recommended since AFP concentrations alone are not reliable indicators of the tumor in fetus and neonate even when results are carefully compared to the age-related RI [1]. On the other hand, in immature teratomas the histological examination of large tumors may show no evidence of YST, even in presence of high serum AFP concentrations [1, 58]. Therefore, in suspected malignant GCT or teratomas the evaluation of serum AFP concentrations prior to surgery is highly recommended for diagnosis as complementary to histological examination [3]. Measurements of AFP concentrations are further required to monitor effects of surgery, chemotherapy and treatment changes [1]. Obviously, the influence of other conditions (e.g. various liver disorders and systemic diseases) on AFP concentrations should be excluded.

Clinical protocols on GCT and teratomas report baseline AFP values ≥10,000 µg/L in >1-year-old patients as predictors of poor outcome [59, 60]. A recent metanalysis mixing pediatric and adult populations has shown that in patients with ovarian YST postoperative AFP concentrations >1000 µg/L may indicate poor prognosis [16].

In intracranial GCT, AFP and hCGβ determinations, both in serum and cerebrospinal fluid (CSF), are required to exclude secreting tumor elements before proceeding to biopsy [6]. Malignant intracranial non-germinomatous GCT (including choriocarcinomas, YST, embryonal carcinomas, and mixed tumors) can be diagnosed without the need for biopsy confirmation if AFP is >25 µg/L and/or hCGβ >50 IU/L in at least one serum/CSF sample (note that these cut-offs have been reported for patients >4 years old) [6, 61]. The same authors reported that serum or CSF AFP concentrations >1000 µg/L have worse prognostic value and require treatment intensification. Other operative protocols have suggested alternative cut-off values

[62–64]. A biopsy is mandated when both AFP and hCGβ in serum and CSF are negative, particularly for distinguishing germinoma from embryonal carcinomas as treatment protocols are different for these malignant subtypes [61].

Coakley et al. [11] reported that in newborns up to 41 weeks of GA, in samples drawn within 24 h from birth, the ratio of plasma AFP concentrations to CSF is approximately 20:1. Using the AxSYM assay (Abbott Diagnostics), they reported that, after correcting for prematurity, AFP CSF 95th percentile concentrations are 889,000 IU/L in infants from birth to 30 days of life and 12,000 IU/L in those aged >5–6 weeks. By the age of 2 months, AFP in CSF should be <3000 IU/L and thereafter be undetectable [11]. The reporting of AFP concentrations in IU/L and the selected age intervals however prevent any comparison with data obtained by other authors on the same biological matrix.

AFP values in the clinical framework

Although available data are relatively few and often controversial, in Table 4 we tried to summarize the rate of AFP positive results according to different tumor histological types [1, 9, 13, 54, 55, 57].

Schneider et al. reported that, in their clinical experience, AFP concentrations >500 μg/L are rarely associated to benign conditions in children [1]. von Schweinitz et al. [53] observed that 10% of HB patients showed AFP concentrations <99 μg/L, 26% AFP from 100 to 9999 μg/L, 56% from 10,000 to 999,999 μg/L, and 8% AFP concentrations >1,000,000 μg/L. In HCC, the available case series reported average AFP values of 200,000 μg/L, with ~20% of results <100 μg/L [54, 55].

Lin et al. [17] in a 10-year case series of pediatric YST and malignant mixed GCT have recorded AFP values up to 74,639 μg/L. Yoshida et al. [12] in YST of infants aged 1 day to 6 months reported average AFP concentrations of 4060 μg/L (range: 64–94,000). Davidoff et al. [14] described a case series of endodermal sinus tumors collected in 22 years of experience (37 patients, aged 5 months–16 years) with preoperative AFP values ranging from 10,000 to 60,000 μg/L. Heerema-McKenney et al. [15], by retrieving a 20-year case series of neonatal and infant congenital teratomas, reported serum AFP concentrations ranging from 15,900 to 170,000 μg/L. Finally, a metanalysis (including data from six trials) on ovarian YST in 78 children aged 0–10 years has recently reported the following incidence of AFP results for range of concentrations: <200 μg/L in 5.1% of cases, 200–1000 μg/L in 6.4%, 1000–10,000 μg/L in 35.9% and >10,000 μg/L in 48.7% of cases, respectively [18].

Table 4: Clinical sensitivity of serum AFP determination in different pediatric tumors.

Tumor type	Rate of AFP positive results[a]
Hepatoblastoma (including epithelial, fetal, embryonal, mixed: epithelial and mesenchymal, and anaplastic)	50%
Hemangioma	14%
Mesenchymal hamartoma	2%
Hepatocellular carcinoma	
Typical	80%
Fibrolamellar	11%
Germ cell tumors	
Yolk sac tumor	>90%
Choriocarcinoma	0%
Dysgerminoma	0%
Embryonal carcinoma	0%
Seminoma	0%
Immature and mature teratoma	<20%

[a]Most authors considered 20 μg/L as threshold value for considering AFP elevated.

The importance of serial serum AFP monitoring and marker HL

Serial serum AFP measurements have been recommended in pediatric patients with GCT for early diagnosis, treatment monitoring and detecting relapse [13, 44, 65–67]. Protocols propose AFP determination 5 days after surgical removal or start of chemotherapy and a retesting interval of 1–2 weeks until normalization, within the 6th month of age of infants [1, 67], or 1 to 3–4 months, within the 3rd year of age, and thereafter every 6 months, until the 5th year of age [44, 45]. In patients with teratomas, a ≤4-month retesting interval enables to guide primary resection of subclinical YST and to detect localized metastases [12]. In HB, three consecutive rising values, occurring at weekly intervals, may be a sign of relapse demanding imaging and histological confirmation [7].

For most authors, demonstration of full response to chemotherapy and/or complete surgical eradication of tumor requires normalization of serum AFP concentrations, even if various thresholds have been used to define 'normalization' [12, 15, 44, 45, 68]. On the other hand, any subsequent elevation or ongoing increase are ominous findings suggestive of recurrent/progressive disease, often anticipating by weeks the evidence by

imaging and requiring prompt changes in management [7]. A too slow AFP decrease may predict a tumor residuum, sometimes being the only evidence of this, and, in cases of YST with chondrosarcoma, the slow decline of AFP concentrations in serum has driven more extensive surgical procedures [69].

According to Ishiguro et al. [65], the serial serum AFP monitoring may contribute more reliable diagnostic information whether the pattern and the extent of the decline are characterized.

As already discussed, there is a consensus on the biphasic pattern of AFP decline referring to the HL of AFP degradation estimated in healthy infants according to age [59, 70]. We retrieved six studies deriving AFP HL from experimental data or by computing regression models (Table 1) [21, 25–28, 33]. Published data were comparable, even if studies using AFP results from serial samples of the same individuals obviously estimated shorter HL than those using single samples from different individuals. Summarizing data, the AFP HL in term babies was approximately

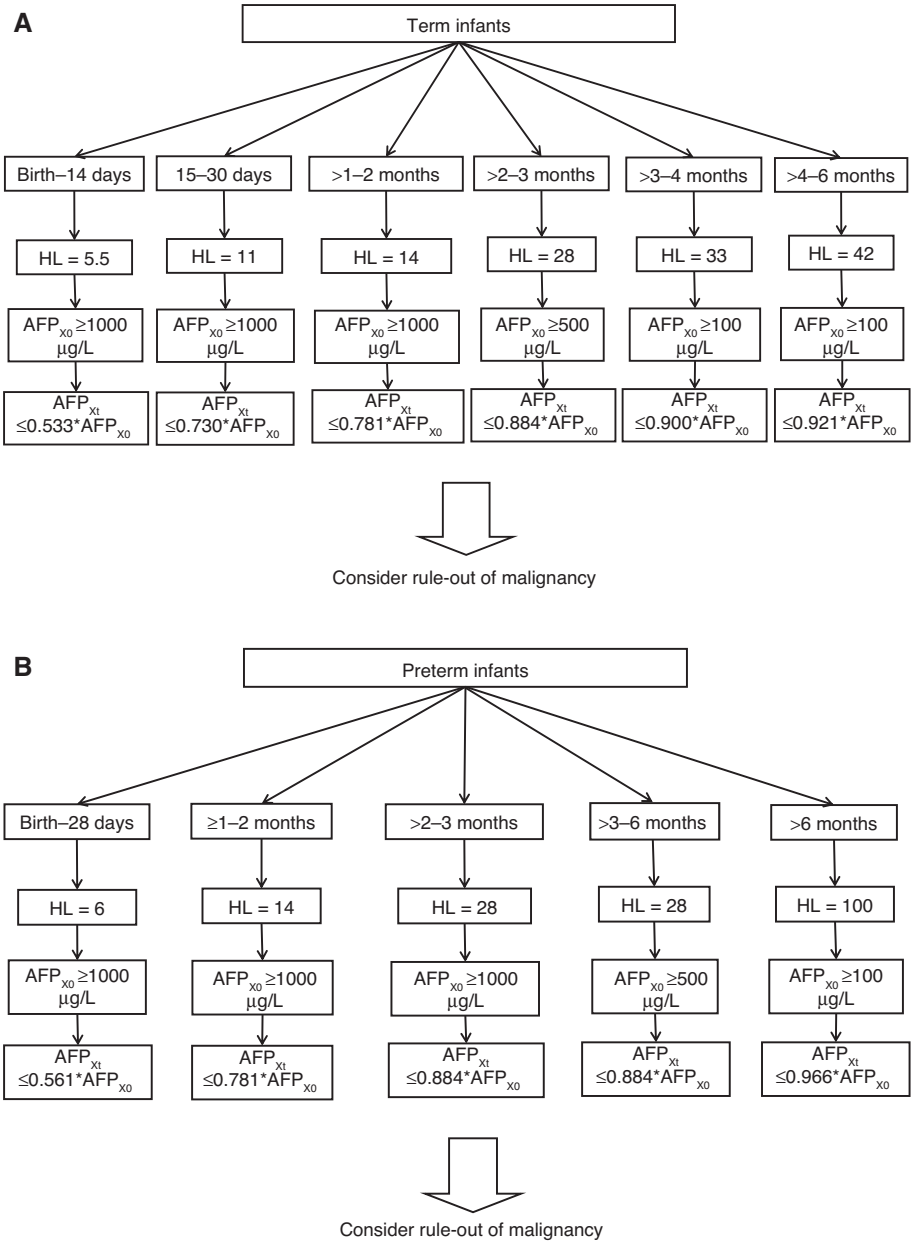

Figure 1: Proposed algorithms for ruling out malignancy according to serum AFP concentrations measured baseline and 5 days later in term (A) and preterm (B) infants of different ages using appropriate marker half-life (HL).
Data for AFP HL (in days) are derived from Refs. [21, 27]. AFP_{x0}, baseline AFP concentration; AFP_{xt}, predicted AFP concentration 5 days later.

5.5 days at birth, 11 days in infants aged 14–30 days, and 33 days up to 4 months of age. Preterm infants appeared to have similar or slightly higher HL for comparable periods. Nevertheless, there was no agreement on the HL thresholds for AFP to be used for treatment monitoring in clinical frameworks and some authors proposed in children with tumors HL values other than those obtained on healthy newborns. For instance, in GCT an AFP decline with a HL ≤4 days was proposed as an effective indicator of remission [65], while a HL <7 days was associated to low risk of recurrence [71]. In children with teratomas, YST and HB, Walhof et al. [56] showed that a linear pattern for AFP decline, with a HL ≤6 days, is suggestive for complete tumor resection by assuming that AFP degradation rate and HL estimated in neonatal conditions are comparable with the situation after radical tumor resection or during effective chemotherapy. Finally, we should not forget that in large GCT and HB spurious AFP increases modulated by therapy cytotoxicity and tumor necrosis may occur [1].

According to the available evidence [1, 12–15, 17, 18], we have summarized in Figure 1 AFP threshold values that, in the first 6 months of life of preterm and term infants, may be associated to a substantial risk of malignancy. In infants with baseline AFP concentrations (i.e. initial pre-treatment AFP values) overcoming these thresholds, a further AFP determination and the interpretation of results according to the HL appropriate for child age should be helpful in improving the clinical information, but this may delay the diagnosis. Assuming a first-order kinetic for AFP disappearance from serum, here we suggest to use the following formula for early predicting AFP concentrations of infants at day 5 after baseline determination, in case they do not have tumors producing AFP: $X_t = X_0 * 2^{-t/HL}$ or $X_t = X_0 * \exp(-0.693 * t/HL)$, where: t = days elapsed for AFP retest (i.e. 5); HL = AFP half-life according to age; X_0 = AFP baseline concentration, and X_t = predicted AFP concentration at day 5. The derived algorithms, as reported in Figure 1, should allow predicting the threshold for AFP concentrations to be used at day 5 for excluding malignancy.

Concluding remarks

The AFP measurement in serum covers an important role in the diagnostic workup and surveillance of relapse of rare GCT, HB and HCC, characterizing fetal, neonatal, infant and pediatric ages. When imaging and histological examinations are not sensitive enough and fail in detecting tumor residuum after surgery or early relapse, and the presence of yolk sac components, clinical guidelines and consensus documents recommend actions to be taken whether AFP results exceed the cut-off appropriate for age and/or AFP concentrations in serum are rising or slowly decreasing according to the marker HL [1–9]. For intracranial GCT, AFP values on serum and CSF above the URL are enough to rule in, while normal AFP requires confirmation by biopsy and when highly increased may guide the intensification of treatment [6, 61]. Importantly, the use of serial AFP determinations for surveillance of relapse has significantly reduced the burden of follow-up, deeply changed the tumor management and increased the 2-year survival rate (currently resulting of about 50%) [14, 45, 65].

Although the interpretative criteria and the clinical efficacy of serum AFP measurement in adult populations have been fully characterized [72], their transfer to pediatric populations may not be equally effective, requiring the development of a *de novo* pediatric classification [73]. In a context of rare tumors, it is undoubtedly of assistance to retrieve indications about AFP results predictive for malignancy from registries, clinical trials and retrospective case series [12–18]. However, with rare exceptions, no indication about the assay used to measure AFP concentrations are available and this represents a major drawback if we consider that most of the evidence derives from case series retrieved in 20–50 years of clinical practice, when AFP assays were not yet harmonized and AFP results were probably not comparable to those obtained by current measuring systems. Accordingly, we may reasonably doubt that AFP concentrations across retrospective studies might be outdated for use in current clinical practice. With the change of assay generations, we are also unaware if the very high AFP concentrations physiologically characterizing the first days of life (from 150,000 to 800,000 μg/L), when measured by previous RIA, can still be detected. More recent studies employing contemporaneous assays for the estimation of RI do not provide AFP measurements in the early neonatal period and, furthermore, the upper limit of measurement after the recommended dilution protocols for these assays is far lower than the above-mentioned concentrations. An additional analytical problem may come from the AFP determination on a not validated biological matrix. In this sense, the AFP measurement in CSF and the derivation of related diagnostic cut-offs have to be considered off-label. This should be strongly remarked, considering that these results may cover a central role in the diagnosis and management of intracranial GCT [6]. The methodological issues affecting AFP determination are substantially disregarded in clinical documents, although the questionable comparability of assays seems the main limitation to the generalization of RI and to interpretation of AFP results at any pediatric

age. Although transference and verification studies are ongoing to expand the applicability of the RI provided in more recent papers [74], novel studies based on robust methodology are warranted to improve the interpretation of AFP results in pediatric oncology.

Further limitations to the reliability of AFP URL quoted by running operative protocols should be ascribed to methodological drawbacks in the design of studies and in the statistical approaches followed for computing data. The low sample size for age partitions, the lack of visual investigation of data, the failure of outlier detection and handling, the assumption of Gaussian distribution of data and the use of parametric approach without preliminarily checking distributions, all may cause significantly biased results [43]. On the other hand, factors unique to pediatric populations (e.g. difficult sample drawing, the presence of interfering conditions, ethical constraints) may strongly limit the correct definition of AFP RI. Several partitions should be warranted in preterm and term newborns in the 1st year of life to capture the striking variability of AFP concentrations in serum, to highlight extrauterine adaptation and development patterns.

Overall, data summarized in this review confirm that the wide variability of available RI is the main limitation to the use of AFP for ruling out malignancy in pediatrics and prevents from the introduction of robust and harmonized URL in the laboratory report, particularly in infants <6 months of age. Unfortunately, most of the tumors requiring diagnosis and surveillance by AFP determination occur within the 1st year of age, with a poorer outcome when presenting in the fetal period [5, 12, 15, 20, 75]. To decrease doubts about the interpretation of AFP results in an infant or child, it has been recommended to repeat the determination after 2–4 weeks and consider the marker kinetic for suspecting malignancy or tumor recurrence [13, 44, 65–67]. A slowly declining pattern or a simply prolonged HL of the marker may indicate a persistent AFP production predictive for the presence of vital tumor tissue (or residual after surgery) [76, 77]. To obtain an early rule out of malignancy in infants with baseline AFP concentrations overcoming the risk thresholds defined for age, we have proposed to check the AFP decline after 5 days resorting to HL appropriate for age (Figure 1). However, this approach warrants further clinical validation in expressly designed studies.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.
Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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