We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

BOOK CITATION INDEX
CLARIVATE ANALYTICS
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Alpha-Fetoprotein in Malignant Pediatric Conditions

Lisa Van Houwelingen and John A. Sandoval

Additional information is available at the end of the chapter

Abstract

Abnormal serum α-fetoprotein (AFP) levels are frequently observed in common disorders such as spina bifida or Down's syndrome in the fetus and cancer in children and adults. The focus of this chapter summarizes on the role of serum α-fetoprotein (AFP) as a useful biomarker in malignant pediatric tumors. The fetal yolk sac and liver generate high levels of AFP during gestation and decline over the next 12 months of infancy, and only trace amounts are detected in childhood. As a result, persistent elevation of AFP correlates with a number of select pediatric malignant conditions. Serum AFP is overexpressed in a considerable fraction of germ cell tumors (GCTs), hepatoblastoma (HB), and hepatocellular carcinoma (HCC). We provide the reader with a review of AFP as a useful specific marker for the diagnosis, management, and follow-up in select pediatric cancers.

Keywords: α-fetoprotein, oncofetal protein, germ cell tumors, hepatoblastoma, hepatocellular carcinoma

1. Introduction

The accurate diagnosis of pediatric solid tumors and the timely recognition of disease recurrence involve the combination of clinical suspicion, imaging techniques, tissue biopsy, and serum tumor markers. Traditional cancer biomarkers are biologically measured substances that are expressed by malignant tissues, or generated by the host in response to the tumor and aid the clinician in diagnosis, staging, assessing response to treatment, and detecting disease recurrence [1]. Tumor markers can be measured in the blood, cerebrospinal fluid, or serous effusions. In general, elevated levels of these biomarkers have been implicated as playing an important role in different types of cancer, and some play a significant role as prognostic indicators [2].

Bergstrand and Czar first identified α-fetoprotein (AFP) in human fetuses in 1956 [3]. Characterized as a glycoprotein that is normally produced during gestation by the yolk sac and the liver during fetal life, AFP is highly elevated in the circulation of newborns, and concentration decreases (half-life, 5 days) to 10–20 µg/L during the first 12 months of infant life [4]. A major mammalian oncofetal protein, AFP is a member of the albuminoid gene superfamily, which consists of AFP, serum albumin, vitamin D-binding protein, and alpha-albumin (afamin) on chromosome 4. AFP is found in monomeric as well as in dimeric and trimeric forms, and binds copper, nickel, fatty acids, and bilirubin [5]. In normal fetuses, AFP binds the hormone estradiol. Altered serum AFP levels have been observed concurrent with aberrant growth manifestation in some birth defects (increased in open neural tube defects, and omphalocele and decreased in Down syndrome) and a subset of endodermal-derived malignancies, most frequently hepatoblastoma (HB), hepatocellular carcinoma (HCC), and germ cell tumors (GCTs), some esophageal and pancreatic carcinomas, and also some benign conditions, particularly those associated with liver damage and regeneration [6]. Given that age-dependent changes in serum AFP levels occur, the consideration of infants' ages while interpreting AFP levels should be underscored. AFP reaches its peak concentration at approximately 12–14 weeks of gestation as this fetal protein is initially synthesized in the yolk sac and then is generated by hepatocytes and gastrointestinal mucosa during embryogenesis. Levels subsequently decline and reach a normal concentration at 8–12 months of age [7]. Because serum AFP concentrations are highly elevated in neonates and exceedingly high levels can be observed in premature infants, AFP levels should be interpreted based on the infant's age and gestational period; normal median levels in term neonates are 41,687 l g/L, and preterm infants, 158,125 l g/L [8].

Due to the age-related changes in AFP values, and the fact that this biomarker is a tumor-associated, not tumor-specific protein, elevation of AFP levels alone does not allow for the diagnosis of malignancy. Additional workup and evaluation including pathologic diagnosis, imaging, and exclusion of other conditions associated with elevations of AFP must compliment tumor marker evaluation. An AFP serum level above 500 µg/L is rarely associated with a benign diagnosis [2].

2. Germ cell tumors

As approximately 50% of GCTs are benign (pure mature teratoma) and do not secrete AFP, the exact incidence of pediatric GCTs is not precisely known. Malignant GCTs account for 3% of all childhood cancers, with 350 new pediatric germ cell tumors diagnosed each year. These tumors develop from variations in normal differentiation (gonadal GCT) and/or aberrant migration (extragonadal GCT) of primordial germ cells. Extragonadal GCTs occur in a variety of midline locations (retroperitoneal, genital, or cranial) but most affect the mediastinum and the sacrococcygeal region. Two groups of GCTs are morphologically divided into seminomas and non-seminomatous germ cell tumors (NSGCTs). Most NSGCTs are a mixture of histologies such as embryonal, choriocarcinomas, teratomas, and yolk sac tumors (YSTs).

Malignant GCTs with yolk sac tumor differentiation are readily characterized with AFP secretion [2]. While predicting behavior in GCT can be confusing secondary to varying factors such as patient age, anatomic site, histologic subtype, and clinical stage, serum levels of AFP are well known in its utility as a noninvasive diagnostic indicator of GCT with yolk sac component [9]. This marker is well validated in its utility as a diagnostic indicator, staging, monitoring of therapeutic response, and subsequent follow-up [10]. In infants and adolescents who are without underlying hepatic disease, a significant elevation of AFP represents a predominant YST component and precludes the diagnosis of a pure mature teratoma or seminoma [1].

The diagnosis of malignant germ cell tumor involves the evaluation of serum AFP in conjunction with the review of histopathology and diagnostic imaging. At times, and often due to sampling error, the pathology may fail to identify a yolk sac component even when the serum AFP is elevated. This is particularly true for intracranial GCT where only limited tissue biopsies are possible [2, 11, 12].

The clinical use of AFP extends beyond initial diagnosis. Tumor marker follow-up examination is also performed during neo-adjuvant chemotherapy to assess for tumor response to therapy. A failure of AFP to decline during chemotherapy may cause clinicians to reevaluate the need for earlier surgical intervention or adjustment of chemotherapy regimen. Prognostic subgroups have been defined based on the ability to evaluate tumor chemotherapy response. For instance, the International Germ Cell Classification Consensus (IGCCC) divides adults into three prognostic groups: good, intermediate, and poor based on the presence of primary tumor, non-pulmonary metastases, and level of tumor biomarkers [10]. Risk-group stratification allows for individual treatment tailoring as well as comparison of results across populations of similarly defined patients and ultimately the collaboration of international clinical trials [13]. This has not yet translated to pediatric oncology as pediatric GCTs are typically divided into risk groups according to stage, histologic grade, and site (gonadal vs. extragonadal). Serum markers have not contributed to risk stratification. The importance of age and level of AFP at diagnosis have varied among studies. A number of groups, including the Children's Cancer and Leukemia Group (CCLG), the French Society of Pediatric Oncology, and Children's Oncology Group (COG), have previously identified serum AFP (>10,000 ng/mL) as a prognostic factor [13–15]. An investigation by the Children's Oncology Group (COG) and the CCLG compared the application of the adult IGCC system to pediatric malignant NSGCT by pooling 25 years worth of clinical trial results. The groups aimed to determine if the tumor biomarker criteria developed in adults would be prognostic among pediatric patients and they also queried whether tumor biomarker data may be relevant in pediatric risk stratification. The results of the study determined that AFP was not prognostic of outcome in pediatric patients, and therefore the utility of AFP in risk stratification in pediatric malignant GCT remains unsettled [16].

Another rare finding while on neo-adjuvant chemotherapy is the growing teratoma syndrome in which a patient with a mixed GCT demonstrates radiographic progression of disease with a reduction of tumor marker. The rationale for continued growth of the tumor is based on the observation that progressive proliferation of the mature or immature components of

the teratoma occurs while the YST component responds to chemotherapy. In this case, prompt surgical excision of the tumor is generally recommended [17, 18].

A final use of AFP as a tumor marker is found following completion of therapy (surgery, chemotherapy, or combination) to assess for the recurrence of disease. The goal after therapy is to have normalization of serum AFP. Tumor recurrence is demonstrated by an abrupt elevation of the AFP level. Yolk sac tumors and mixed germ cell tumors require close follow-up with serial AFP level monitoring as part of the surveillance strategy for this patient population.

3. Hepatoblastoma and hepatocellular carcinoma

Approximately 100–150 new cases of primary liver malignancies are diagnosed annually in the USA and represent for slightly more than 1% of all pediatric tumors [19]. Hepatoblastoma (HB), hepatocellular carcinoma (HCC), sarcomas, germ cell tumors, and rhabdoid tumors constitute the spectrum of malignant liver tumors seen in children. The majority of these are embryonal HB, which accounts for over half (66%) of malignant hepatic neoplasms.

Serum AFP levels are an important tumor marker for HB and HCC. In addition, serum AFP levels may also be elevated in other nonmalignant states such as mesenchymal hamartoma, infantile hepatic hemangioendothelioma, cirrhosis, viral and chronic active hepatitis, tyrosinemia, ulcerative colitis, and in various immune-deficiency conditions [20, 21].

Most children with HB have extremely elevated serum AFP levels (up to $100,000 \pm 1,000,000$ times the normal value (<10 ng/mL)), but roughly 5–10% of patients have unexpectedly low or even normal AFP levels [22]. For HB, serum AFP level is a required diagnostic test, given the correlation between AFP level and tumor burden. Additionally, serial determinations of AFP determine the effectiveness of therapy as AFP concentrations decline to normal levels with effective therapy and persistently elevated levels suggest residual disease. The half-life of the circulating oncoprotein in the bloodstream is between 4 and 9 days, and levels usually return to the normal range (<10 ng/mL) by 4 ± 6 weeks of complete resection [23, 24]. Rising levels following surgery suggest disease progression, recurrence, incomplete tumor resection, or metastases.

Serum AFP is also used as a screening tool to detect the development of HB in children with Beckwith-Wiedemann syndrome (BWS) or isolated hemihyperplasia (HH). The development of HB in these cancer predisposition syndromes has been observed in 1–3% of patients with BWS and HH. Improved survival is dependent on early detection. This can be inferred by the improved prognosis of early stage (stage I and II) HB as compared to late stage (stage III and IV) tumors: 91% of 5-year event-free survival vs. 25–64% of 5-year event-free survival, respectively. This is accomplished by routine screening with hepatic ultrasound and serum AFP at 3-month intervals up to the age of 4 years [25].

A final use of serum AFP in children with HB is its use in prognostication. Although complete surgical resection in HB is the best predictor of survival, more than half of children

initially present with advanced unresectable disease (stage III or IV, North American Staging System). In an effort to achieve cytoreduction and improved resectability, children with up-front unresectable HB receive neo-adjuvant cisplatin-based chemotherapy [26]. HB patients who are poor responders to induction therapy (as demonstrated by persistently elevated AFP levels) have been shown to have a significantly worse outcome [23]. In fact, the strongest predictor of survival in unresectable or metastatic HB is the timing and significance of the AFP level change during neo-adjuvant chemotherapy administration. A large early decrease in AFP level correlates with the best outcome. It has been further suggested that monitoring the changes in AFP during neo-adjuvant chemotherapy may help identify poor responders and, in those patients, we should consider a change in therapeutic approach [27].

Another poor predictor of survival is low serum AFP at diagnosis. Although most patients present with elevated levels, serum AFP is low or normal in approximately 5–10% of cases. Children with HB and low AFP level at diagnosis have been identified as a high-risk group. This group of patients will generally present with more advanced disease at diagnosis and have a high degree of treatment failure [28].

HB can also be linked with the abnormal secretion of peptide hormones and other proteins. Marked thrombocytosis secondary to the release of tumor-derived elements with growth factors, thrombopoietin-like activity, platelet-derived microparticles, components released from bone marrow endothelial cells, and growth-related factors secreted by megakaryocytes can impact these biologic processes [29–31]. Likewise, ectopic beta subunit of human chorionic gonadotropin (βHCG) secretion can lead to precocious puberty in boys (virilizing HB) or a *forme fruste* in girls [32]. Grunewald and scholars reported a case study of a hormonally active HB causing both ectopic adrenocorticotropic hormone (ACTH) syndrome and parathyroid hormone (PTH)-related peptide-induced hypercalcemia [33]. While these paraneoplastic syndromes are not commonly associated to HB, they stress the importance of considering these oncofetal antigens as tumor-associated rather than tumor-specific, and the varied clinical presentations that can pose further diagnostic and therapeutic challenges in HB.

HCC is the second most common hepatic malignancy in children and accounts for less than 0.5% of all pediatric cancer [34]. An estimated 0.5–1.0 cases per million children is the reported relative frequency for HCC [35]. Unlike HB, it is more commonly found in older children and teenagers with underlying liver conditions (Alagille syndrome, viral hepatitis B/C, progressive familial intrahepatic cholestasis, inborn errors of metabolism [tyrosinemia and glycogen storage diseases I–IV]). The 5-year overall survival (OS) rate is 42% for children and adolescents with hepatocellular carcinoma. The use of AFP levels in HCC has been primarily for diagnostic purposes. Most HCCs are associated with elevated levels of AFP. Although not found to be an independent predictor of survival, it has been observed that HCCs with serum AFP of <100 ng/mL at diagnosis tend to have a worse outcome and are frequently observed in the fibrolamellar HCC variant [36]. Serum AFP may also be used for surveillance purposes in the "at-risk" patient. Patients treated for biliary atresia (obstructive cholangiopathy of the newborn) in infancy should be monitored for the development of cirrhosis and subsequently HCC as well as cholangiocarcinoma. This can be accomplished with abdominal ultrasound and serum AFP levels at 6–12-month intervals [37]. Children who are diagnosed with

inborn errors of metabolism, congenital malformations, or who have cirrhosis induced by chronic liver disease require screening for the development of HCC.

4. AFP and associated rare pediatric solid cancers

While serum AFP is primarily used in the diagnosis and management of GCT (with yolk sac component), HB, and HCC, elevated levels have also been found in select cases of nephroblastoma (Wilms tumor, WT) and pancreatoblastoma (PB). For example, Roth and colleagues first described WT presenting with elevated serum AFP [38]. Since this initial description, only four other WT cases have been documented with histologically classic type WT and elevated AFP [39–41]. More notably, elevated AFP is seen in the teratoid WT, in which more than 50% of the tumor is composed of teratoid elements [42]. Nevertheless, due to the extremely rare occurrence, serum AFP is not a typical tumor marker for pediatric renal tumors.

PB is a rare childhood malignancy with only 62 cases reported in the literature [43]. It has been described as an embryonal hamartomatous neoplasm arising from multipotential stem cells [44]. Typically presenting as an abdominal mass with progressive jaundice, PB has been associated with an elevated serum AFP at diagnosis in 25% of the described cases. While clinically affiliated with a prolonged, indolent course with favorable outcomes, serum AFP may contribute to the diagnosis and monitoring of therapy for PB [45].

5. Conclusion

Although AFP is a well-known classical serum biomarker that can assist with diagnosis of certain malignant solid tumors in children, it remains limited in sensitivity and specificity [46]. Other promising biomarkers for tumor diagnosis, especially GCT, include microRNAs (miRNAs), which are short nucleic acid molecules synthesized in the cellular nucleus. miRNAs modify posttranscriptional gene activity by targeting mRNA molecules. Once released from the nucleus, miRNAs can enter almost all biological fluids (e.g., serum, saliva, urine, and milk), where they are thought to mediate intercellular communication. After departure from the cell of origin, miRNAs show remarkable stability because of their inclusion in membrane vesicles (exosomes). As a result, blood-based miRNAs show considerable promise for cancer diagnosis and monitoring [47]. miRNAs encoded by the small miR-371-3 cluster that maps to the terminal region of the long arm of chromosome 19 are among the most promising candidates for diagnosing and monitoring malignant GCTs, as these tumors overexpress the miR-371-373 and miR-302/367 clusters, regardless of the age of the patient, the histological subtype of the tumor, or its anatomical site [47]. Murray et al. have reviewed the current understanding of miRNAs and their role in diagnosing and monitoring childhood GCTs [48]. The applicability of miRNAs for the screening and diagnosis to clinical decision-making aids, surveillance biomarkers, or sources of real-time molecular characterization may emerge as the next generation "smart" biomarkers for pediatric solid malignancies.

Author details

Lisa Van Houwelingen[1] and John A. Sandoval[2*]

*Address all correspondence to: john.sandoval@bmg.md

1 St Jude Children's Research Hospital, Memphis, TN, USA

2 Baptist Children's Hospital, Memphis, TN, USA

References

[1] Sandoval, J.A.; Malkas, L.H.; Hickey, R.J. Clinical Significance of Serum Biomarkers in Pediatric Solid Mediastinal and Abdominal Tumors. *Int J Mol Sci* 2012; 13:1126–1153.

[2] Schnieder, D.T.; Calaminus, G.; Gobel, U. Diagnostic Value of Alpha-Fetoprotein and Beta-Human Chorionic Gonadotropin in infancy and Childhood. *Pediatr Hematol Oncol* 2001; 18: 11–26.

[3] Bergstrand C.G.; Czar B. Demonstration of a New Protein Fraction in Serum from the Human Fetus. *Scand J Clin Lab Invest* 1956; 8: 174.

[4] Blohm, M.E.; Vesterling-Horner, D; Calaminus,G; Gobel, U. Alpha 1-Fetoprotein (AFP) Reference Values in Infants up to 2 Years of Age. *Pediatr Hematol Oncol* 1998; 15(2): 135–142.

[5] Aoyagi, Y.; Ikenaka, T.; Ichida, F. alpha-Fetoprotein as a Carrier Protein in Plasma and its Bilirubin-Binding Ability. *Cancer Res* 1979; 39(9):3571–3574.

[6] Marshall, W.; Bangert, S. Clinical Biochemistry: Metabolic and Clinical Aspects. 2nd ed. Philadelphia, PA: Churchill Livingstone, Elsevier; 2008.

[7] Mueller, B.U.; Lopez-Terrada, D., Finegold, M.J. Tumors of the Liver. In *Principles and Practices of Pediatric Oncology*, 5th ed; Pizzo, P., Poplack, D., Eds. Philadelphia, PA: Lippincott Williams & Wilkins., 2006; pp. 887–904.

[8] Wu, J.T.; Book, L.; Sudar, K. Serum alpha-Fetoprotein (AFP) Levels in Normal Infants. *Pediatr Res* 1981; 15: 50–52.

[9] Billmire, D., Vinocur, C., Rescorla, F. et al. Outcome and Staging Evaluation in Malignant Germ Cell Tumors of the Ovary in Children and Adolescents: An Intergroup Study. *J Pediatr Surg* 2004; 39(3): 424–429.

[10] International Germ Cell Cancer Collaborative Group. International Germ Cell Consensus Classification: A Prognostic Factor-based Staging System for Metastatic Germ Cell Cancers. *J Clin Oncol* 1997; 15: 594–603.

[11] Gobel, U.; Calaminus, G.; Engert, J. Teratomas in Infancy and Childhood. *Med Pediatr Oncol* 1998; 31: 8–15.

[12] Harms, D.; Janig, U. Germ Cell Tumors of Childhood: Report of 170 Cases Including 59 Pure and Partial Yolk Sac Tumors. *Virchows Arch A* 1986; 409: 223–239.

[13] Frazier, A.L.; Rumcheva, P.; Olson,T.; Giller, R.; Cushing, B.; Cullen, J.; Marina, N.; London, W.B. Application of the Adult International Germ Cell Classification System to Pediatric Malignant Non-Seminomatous Germ Cell Tumors: A Report from the Children's Oncology Group. *Pediatr Blood Cancer* 2008; 50: 746–751.

[14] Mann, J.R.; Raafat, F.; Robinson, K.; Imerson, J.; Gornall, P.; Sokal, M.; Gray, E.; McKeever, P.; Hale, J.; Bailey, S.; Oakhill, A. The United Kingdom Children's Cancer Study Group's Second Germ Cell Tumor Study: Carboplatin, Etoposide, and Bleomycin are Effective Treatment for Children with Malignant Extracranial Germ Cell Tumors, with Acceptable Toxicity. *J Clin Oncol* 2000; 18: 3809–3818.

[15] Baranzelli, M.C.; Kramar, A.; Bouffet, E.; Quintana, E.; Rubie, H.; Edan, C.; Patte, C. Prognostic Factors in Children with Localized Malignant Nonseminomatous Germ Cell Tumors. *J Clin Oncol* 1999; 17: 1212.

[16] Frazier, A.L.; Hale, J.P.; Rodriguez-Galindo, C.; Dang, H.; Olson, T.; Murray, M.J.; Amatruda, J.F.; Thornton, C.; Arul, G.S.; Billmire, D.; Shaikh,F.; Farzana,P.; Stoneham, S.; Krailo, M.; Nicholso, J.C. Revised Risk Classification for Pediatric Extracranial Germ Cell Tumors Based on 25 Years of Clinical Trial Data From the United Kingdom and United States. *J Clin Oncol* 2015; 33:195–201.

[17] Chen LT, Chen CL, Hwang WS. The growing teratoma syndrome. A case of primary mediastinal nonseminomatous germ cell tumor treated with chemotherapy and radiotherapy. *Chest*. 1990 Jul;98(1):231–3.

[18] Afifi HY, Burt ME. Mediastinal growing teratoma syndrome. *Ann Thorac Surg*. 1997 Aug;64(2):359–62.

[19] Bulterys, M.; Goodman, M.T.; Smith, M.; Buckley, J.D. Hepatic Tumors. In Cancer Incidence, Survival Among Children, Adolescents: United States SEER Program 1975–1995. Available online: http://seer.cancer.gov/publications/childhood/ (access on 12 November 2015).

[20] Urbach, A.H.; Zitelli, B.J.; Blatt, J.; Gartner, J.C.; Malatack, J.J. Elevated alpha-Fetoprotein in a Neonate with Benign Hemangioendothelioma of the Liver. *Pediatrics* 1987; 80: 596–597.

[21] Sari, N.; Yalcin, B.; Akyuz, C.; Halioglu, M.; Buyukpamukcu M. Infantile Hepatic Hemangioendothelioma with Elevated Serum alpha-Fetoprotein. *Pediatr Hematol Oncol* 2006; 23: 639–647.

[22] Kelli, S. Serum Markers in Tumor Diagnosis and Treatment. In *Paediatric Oncology-Clinical Practice and Controversies*, 3rd ed; Pinkerton, R., Plowan, P.N., Pieters, R., Eds. London, UK: Edward Arnold Publishers, 2004; pp. 169–188.

[23] Van Tornout, J.M.; Buckley, J.D.; Quinn, J.J; Freusner, J.H.; Krailo, M.D.; King, D.R.; Hammond, G.D.; Ortega, J.A. Timing and Magnitude of Decline in alpha-Fetoprotein Levels in Treated Children with Unresectable or Metastatic Hepatoblastoma are Predictors of Outcome: A Report from the Children's Cancer Group. *J Clin Oncol* 1997; 15: 1190–1197.

[24] Lovvorn, H.N. III; Ayers, D.; Zhao, Z.; Hilmes, M.; Prasad, P.; Shinall, M.C., Jr.; Berch, B.; Neblett, W.W., III; O'Neill, J.A., Jr. Defining Hepatoblastoma Responsiveness to Induction Therapy as Measured by Tumor Volume and Serum alpha-Fetoprotein Kinetics. *J Pediatr Surg* 2010; 45: 121–128.

[25] Clericuzio, C.L.; Chen, E.; McNeil, D.E.; O'Connor, T.; Zackai, E.; Medne, L.; Tonlinson, G.; DeBaun, M. Serum alpha Fetoprotein Screening for Hepatoblastoma in Children with Beckwith-Wiedemann Syndrome or Isolated Hemihyperplasia. *J Pedaitr* 2003; 143(2): 270–272.

[26] Ortega, J.A.; Douglass, E.C.; Feusner, J.H.; Reynolds, M.; Quinn, J.J.; Finegold, M.J.; Haas, J.E.; King, D.R.; Liu-Mares, W.; Sensel, M.G.; Krailo, M.D. Randomized Comparison of Cisplatin/Vincristine/Fluoruracil and Cisplatin/continuous Infusion Doxorubicin for Treatment of Pediatric Hepatoblastoma: A Report from the Children's Cancer Group and the Pediatric Oncology Group. *J Clinl Oncol* 2000; 18(14): 2665–2675.

[27] Sayar, D.; Yaniv, I.; Goshen, Y.; Cohen, I.J. Treatment of alpha-Fetoprotein Secreting Hepatoblastoma by Response of Serum alpha-Fetoprotein Levels: A New Concept. *Pediatr Hematol Oncol* 2001; 18: 509–518.

[28] De Ioris, M.; Brugieres, L.; Zimmermann, A.; Keeling,J.; Brock, P.; Maibach, R.; Pritchard, J.; Shafford, L.; Zsiros, J.; Czaudzerna, P.; Perilongo, G. Hepatoblastoma with a Low Serum alpha-Fetoprotein Level at Diagnosis: The SIOPEL Group Experience. *Eur J Cancer* 2008; 44: 545–550.

[29] Nickerson, H.J.; Silberman, T.L.; McDonald, T.P. Hepatoblastoma, Thrombocytosis, and Increased Thrombopoietin. *Cancer* 1980; 45: 315–317.

[30] Shafford, E.A.; Pritchard, J. Extreme Thrombocytosis as a Diagnostic Clue to Hepatoblastoma. *Arch Di. Child* 1993; 69: 171.

[31] Komura, E.; Matsumura, T.; Kato, T.; Tahara, T.; Tsunoda, Y.; Sawada, T. Thrombopoietin in Patients with Hepatoblastoma. *Stem Cells* 1998; 16: 329–333.

[32] Perilongo, G.; Rigon, F.; Murgia, A. Oncologic Causes of Precocious Puberty. *Pediatr Hematol Oncol* 1989; 6: 331–340.

[33] Grunewald, T.G.; von Luettichau, I.; Welsch, U.; Dorr, H.G.; Hopner, F.; Kovacs, K.; Burdach, S.; Rabl, W. First Report of Ectopic ACTH Syndrome and PTHrP-induced

Hypercalcemia Due to a Hepatoblastoma in a Child. *Eur J Endocrinol* 2010; 162: 813–818.

[34] Czauderna, P.; Mackinlay, G.; Perilongo, G.; Brown, J.; Shafford, E.; Aronson, D.; Pritchard, J.; Chapchap, P.; Keeling, J.; Plaschkes J.; Otte, J.B. Hepatocellular Carcinoma in Children: Results of the First Prospective Study of the International Society of Pediatric Oncology Group. *J Clin Oncol* 2002; 12: 2798–2804.

[35] Bellani, F.F.; Massimino, M. Liver Tumors in Childhood: Epidemiology and Clinics. *J Surg Onc* 1993; Suppl 3: 119–121.

[36] Walther, A.; Tiao, G. Approach to Pediatric Hepatocellular Carcinoma. *Clin Liver Dis* 2013; 2: 219–222.

[37] Fukuda, A.; Sakamoto, S.; Kanazawa, H.; Shigeta, T.; Karaki, C.; Hamano, I.; Uchida, H.; Kitagawa, U.; Okuse, C.; Miyazaki, O.; Nosaka, S.; Nakazawa, A.; Kasahara, A. Incidentally Detected Cholangiocarcinoma in an Explanted Liver with Biliary Atresia after Kasai Operation. *Pediatr Transplant* 2013; E: 62–66.

[38] Roth, A. An Unusual Renal Tumor, Associated Nephroblastoma, Nephroblastomatosis, Teratoma, and Cystic Dysplasia with Arterial Hypertension and Secretion of alpha-Fetoprotein. *J Urol (Paris)* 1984; 90: 7–10.

[39] Erol, K.; Vedat, K.; Erkanm D.; Mustafa, G.; Duygu, H.; Avni, A.A. Wilms Tumor Associated with Elevated alpha-Fetoprotein Level. *Pediatr Blood Cancer* 2005; 44: 423–424.

[40] Patriarca, C.; Orazi, A.; Massimino, M.; Luksch, R. A Cystic Partially Differentiated Nephroblastoma Producing alpha-Fetoprotein. *Am J Pediatr Hematol Oncol* 1992; 12: 352–355.

[41] Crocoli, A.; Madafferi, S.; Jenkner, A.; Zaccara, A.; Inserra, A. Elevated Serum alpha-Fetoprotein in Wilms Tumor May Follow the Same Pattern of Other Fetal Neoplasms After Treatment: Evidence from Three Cases. *Pediatr Surg Int* 2008; 24: 499–502.

[42] Dhungel, S.; Cheng, L.J.; Hai, Z.Z. Elevated Serum alpha-Fetoprotein in Wilms' Tumor: A Case Report with Review of Literature. *J Pediatr Surg Case Rep* 2014; 2: 153–155.

[43] Chan, M.H.M.; Shing, M.M.K.; Poon, T.C.W.; Johnson, P.J.; Lam, C.W.K. alpha-Fetoprotein Variants in A Case of Pancreatoblastoma. *Ann Clin Biochem* 2000; 37: 681–685.

[44] Iseki, M.; Suzuki, T.; Koizumi, Y.; Hirose, M.; Laskin, W.B.; Nakazawa, S.; Ohaki, Y. Alpha-Fetoprotein-Producing Pancreatoblastoma. *Cancer* 1986; 57: 1833–1835.

[45] Morohoshi, T.; Sagawa, F.; Mitsuya, T. Pancreatoblastoma with Marked Elevation of Serum alpha-Fetoprotein. *Virchows Archiv A Pathol Anat* 1990; 416: 265–270.

[46] Murray, M.J.; Nicholson, J.C. α-Fetoprotein. *Arch Dis Child Educ Pract Ed* 2011; 96: 141–147.

[47] Murray, M.J.; Halsall, D.J.; Hook, C.E.; Williams, D.M.; Nicholson, J.C.; Coleman, N. Identification of microRNAs rom the miR-371-373 and miR-302 Clusters as Potential Serum Biomarkers of Malignant Germ Cell Tumors. *Am J Clin Pathol* 2011; 135:119–125.

[48] Murray, M.J.; Nicholson, J.C.; Coleman, N. Biology of Childhood Germ Cell Tumour, Focusing on the Significance of microRNAs. *Andrology* 2014.doi: 10.111/andr.277