JBUON 2013; 18(4): 1026-1031
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

# Clinicopathological features and management of hepatic vascular tumors. A 20-year experience in a Greek University Hospital

T. Theodosopoulos[1], D. Dellaportas[1], A. Tsangkas[2], N. Dafnios[1], V. Psychogiou[1], A. Yiallourou[1], G. Polymeneas[1], A. Kondi-Pafiti[2]

[1]2nd Department of Surgery, [2]Department of Pathology, Aretaieion University Hospital, Athens, Greece

## Summary

***Purpose:*** *The aim of this study was to review the clinical features, diagnostic modalities, treatment options and pathological characteristics of various types of hepatic vascular tumors treated in our Institution over the last 20 years.*

***Methods:*** *From 1991 to 2011, 761 cases of various hepatic lesions, benign and malignant, were surgically treated in our hospital. Among these, 56 (7.35%) hepatectomy specimens referred to vascular tumors. The records of these patients were retrieved and demographics , tumor characteristics, treatment, and actuarial survival were analyzed. The various therapeutic procedures, postoperative complications, follow-up data and the pre-and postoperative diagnostic difficulties were registered and analyzed.*

***Results:*** *Pathological examination showed: hepatic hemangiomas in 35 (62.5%) patients (80% females), hepatic angiosarcomas in 7 patients (12.5%; males 71.4%), hepatic epithelioid hemangioendotheliomas in 9 (16%; females 50%) and hepatic angiomyolipomas in 5 patients (9%; females 60%).*

***Conclusions:*** *Vascular tumors of the liver comprise a heterogeneous group of neoplasms, benign, malignant and of intermediate degree of malignant behavior. These lesions can create great diagnostic difficulties, pre– and postoperative considerations, but the correct pathological diagnosis and classification of vascular hepatic tumors are most of the times mandatory in order to choose the proper therapeutic actions.*

***Key words:*** *angiomyolipomas, angiosarcoma, epithelioid hemangioendotheliomas, hemangioma, liver tumors*

## Introduction

Primary hepatic tumors arise from the different components of the liver, mainly from the hepatocytes and the bile duct epithelium and rarely from neuroendocrine cells and mesenchymal cells which are a minor component of liver histology. The mesenchymal tumors of the liver are rare and may exhibit vascular, fibrous, adipose, and other mesenchymal tissue differentiation.

In this study we report our 20-year experience of primary vascular liver tumors, which comprise a small subgroup among varying pathologies in hepatic lesions [1]. Most common hepatic vascular tumors are the hepatic hemangiomas, which constitute the most common benign liver tumor, occurring in up to 7% of the adult population [2]. The unusual epithelioid hemangioendotheliomas, angiomyolipomas and hepatic angiosarcomas are also encountered and present additional interest because of the diagnostic difficulties during the pre-operative work-up as well as the pathological examination. In the current study we reviewed the clinical features, diagnostic modalities, treatment options and pathological characteristics of various types of hepatic vascular tumors treated in our Institution.

*Correspondence to:* Dionysios Dellaportas, MD. 2nd Department of Surgery, University of Athens, Aretaieio Hospital, 76 Vassilissis Sofias Ave., 11528, Athens, Greece. Tel: +30 6947718723, Fax: +30 210 7286128, E-mail: dellapdio@gmail.com
Received: 16/12/2012; Accepted: 18/01/2013

## Methods

A retrospective review of the files of patients treated for vascular hepatic tumors in our hospital during a 20-year period was carried out. Patient demographics, tumor characteristics, treatment, and actuarial survival were retrieved and analyzed. The various therapeutic procedures, postoperative complications, follow-up were registered as well as the pre-and postoperative diagnostic difficulties.

*Statistics*

As this was a descriptive study, no actual comparison of data between groups was done. Thus, only basic descriptive analysis was performed using SPSS for Windows, version 17.0.

## Results

From 1991 to 2011, 761 cases of various hepatic lesions, benign and malignant, were surgically treated. Among these, 56 (7.35%) cases of hepatic vascular tumors were found. Analysis of the relevant data provided the following results (Table 1).

*Hepatic hemangiomas*

Seven patients (20%) were male (mean age 52 years, range 48-62) and 28 (80%) female (mean age 46 years, range 43-50). The main clinical findings were hepatomegaly, vague abdominal pain and discomfort and palpable upper abdominal mass (Table 2). In one case (32-year-old female using oral contraceptives) signs of acute surgical abdomen due to spontaneous rupture of a liver hemangioma was observed. Preoperative imaging investigations in most cases started using abdominal ultrasound scan, which was followed by abdominal computed tomography (CT scan), and in difficult diagnostic cases liver magnetic resonance imaging (MRI) was finally performed. Although asymptomatic hemangiomas should be ignored, our reported cases were mostly symptomatic due to their large size, and in 3 cases, where the preoperative diagnosis was questionable, an exploratory laparotomy and resection for pathology study was decided.

Pathological examination showed that in 25 cases tumors were large (>7 cm), with mean tumor diameter about 9 cm, mostly solitary (34 cases; 97%) and in one case a diffuse angiomatous lesion occupying all the excised hepatic tissue was observed. Tumors were located mainly in the right lobe (N=29; 83%). Histologically 23 cases (91.4%) were of the cavernous hemangioma variety, while 3 cases consisted of mixed capillary and cavernous hemangiomas. Various degrees of fibrosis were observed in the larger tumors, as well as microcalcifications at the fibrous septa separating vascular spaces.

Open surgical approach was performed and consisted of enucleation in most patients (30 cases; 85.7%) which is the preferred option in order to preserve liver parenchyma. In 4 (11.4%) cases a non-formal right hepatectomy was chosen due to the deep location of the lesion in the liver parenchyma, and one case was treated with right hepatectomy due to the large size of the lesion, which was safely removed by a more extensive procedure. Postoperative complications developed in 6 (17.1%) patients (Table 3). Two had pleural effusions which were treated with thoracentesis and evacuation and 1 had a subcapsular hematoma, treated conservatively. One patient developed urinary tract infection, and finally 2 were treated for postoperative respiratory infection. All patients remained well during a follow up period of 2-4 years after surgery. Overall lesion size and symptoms (abdominal discomfort and pain) were correlated directly, as lesions measuring more than 8 cm caused more severe symptomatology, and also location on the left lateral liver segment could provoke early satiety and abdominal fullness after meals.

*Hepatic angiosarcoma*

This tumor was discovered in 7 (12.5%) patients, 5 (71.4%) male and 2 (28.6%) female, aged from 55 to 62 years. No thorotrast exposure or exposure to vinyl chloride or arsenic compounds

**Table 1.** Clinocopathological classification of 56 cases of hepatic vascular tumors

| *Tumor type* | *Males N (%)* | *Females N (%)* | *Age, years, median (range)* | *Mean diameter (cm, range)* |
|---|---|---|---|---|
| Hemangioma | 7 (12.5) | 28 (50) | 52 (43-62) | 8.5 (4-12) |
| Angiosarcoma | 5 (8.9) | 2 (3.5) | 57 (55-62) | 8 (8) |
| Epithelioid hemangioendothelioma | 4 (7.1) | 5 (8.9) | 35 (28-47) | 5 (4-6) |
| Epithelioid angiomyolipoma | 2 (3.5) | 3 (5.4) | 47 (32-55) | 3.1 (2-4) |
| Total | 18 | 38 | | |

**Table 2.** Main clinical findings at presentation

| *Tumor type/Clinical findings* | *No symptoms/ No clinical findings* | *Hepatomegaly* | *Distinct palpable mass* | *Abdominal discomfort/pain* |
|---|---|---|---|---|
| | *N (%)* | *N (%)* | *N (%)* | *N (%)* |
| Hemangioma | 3 (8.6) | 1 (2.9) | 2 (5.7) | 29 (82.9) |
| Angiosarcoma | 2 (28.5) | 0 (0) | 2 (28.5) | 3 (42.9) |
| Epithelioid hemangioendothelioma | 5 (55.5) | 1 (11.1) | 1 (11.1) | 2 (22.2) |
| Epithelioid angiomyolipoma | 3 (60) | 0 (0) | 1 (11.1) | 1 (20) |
| Total | 13 | 2 | 6 | 35 |

**Table 3.** Postoperative complications

| *Tumor type/Postoperative complication* | *Respiratory infection* | *Pleural effusion* | *Biloma* | *Urinary tract infection* | *Hematoma* |
|---|---|---|---|---|---|
| | *N* | *N* | *N* | *N* | *N* |
| Hemangioma | 2 | 2 | 0 | 1 | 1 |
| Angiosarcoma | 0 | 0 | 1 | 1 | 0 |
| Epithelioid hemangioendothelioma | 1 | 0 | 0 | 0 | 1 |
| Epithelioid angiomyolipoma | 1 | 0 | 1 | 1 | 0 |
| Total, N (%) | 4 (7.1) | 2 (3.5) | 2 (3.5) | 3 (5.4) | 2 (3.5) |

(pecticides) was mentioned in 4 of these cases, and no available data existed for the remaining 3 cases. In all cases there were signs of severe liver disease, liver enlargement, ascites and abdominal pain. Preoperative diagnosis was not accurately made by imaging modalities and open surgery was decided. Formal right hepatectomy was performed in 2 cases, and formal left hepatectomy in 1 case, right trisegmentectomy in 1 case, while atypical liver resections were done in the 3 remaining patients. A biloma, treated conservatively with percutaneous drainage under CT guidance, was the most important postoperative complication recorded. Pathological examination showed that all tumors were large (mean diameter 8 cm) and in 2 cases occupied the whole of the specimen examined. Microscopic examination showed features of typical hepatic angiosarcoma in 3 cases, fibrosarcoma-like angiosarcoma affected 2 cases and Kaposi-like angiosarcoma in the last 2 cases. Immunohistochemistry was an invaluable aid in the differential diagnosis and the neoplastic cells expressed positive immunoreaction to CD31, CD34 and Factor VIII.

All of these patients died 8-16 months after surgery.

*Hepatic epithelioid hemangioendothelioma*

This tumor was diagnosed in 9 (16%) patients, 5 women and 4 men, with age range from 28 to 47 years. Clinical signs were upper abdominal pain and postprandial discomfort, nausea and hepatomegaly, which led to imaging investigations. Abdominal ultrasound and CT scan did not establish a definite preoperative diagnosis, thus surgical excision was the chosen diagnostic option. The basic therapeutic modality was surgical resection with a non-formal hepatectomy or wedge resection. Pathological examination showed that the tumors were multifocal in 4 (44.4%) cases and measured 4-6 cm. In one case, a hepatic peri-portal fibrosis and in another case cirrhotic changes of the liver were observed. The differential diagnosis from cholangiocarcinoma was the most prominent diagnostic problem encountered. Positive immunoreaction of the tumor cells to CD31, CD34 and factor VIII was a valuable diagnostic aid.

No information about adjuvant therapy was available. Two (22.2%) patients developed disease recurrence (in the liver and the retroperitoneal space) and 3 patients survived 2-4 years after diagnosis, while 4 patients were lost to follow up.

*Hepatic angiomyolipoma*

This tumor was diagnosed in 5 (9%) cases, 3 (60%) females and 2 (40%) males, with a mean age of 54 years. Three patients were asymptomatic and the hepatic lesion was revealed during an unrelated imaging examination, while 2 presented with vague abdominal discomfort/pain and

were investigated with abdominal CT scan. Segmentectomies and atypical liver resections were performed in all cases and no significant postoperative complications were observed. In 3 cases gross examination showed the lesion located in the right lobe and the size of lesions ranged from 2 to 4 cm; all lesions were solitary. Histologically, the tumors were mainly composed of smooth muscle tissue, adipose tissue and thick-walled vessels with narrowing or obliteration of their lumen. All patients were disease-free 3-5 years after surgical extirpation of the lesion. No additional therapy was proposed. In general, angiomyolipomas should not be treated surgically, but these 2 patients complained of symptoms that preoperative investigations could not relate to other diseases and the remaining 3 had no definite diagnosis by imaging modalities.

## Discussion

Hemangiomas of the liver are found in 7% of the general population, affecting more commonly women and remain mostly asymptomatic. Giant hepatic hemangiomas (> 4cm) are symptomatic in 40% of the cases. Clinical manifestations are rare and may include abdominal pain, early satiety, anorexia, and nausea. Their pathogenesis is controversial, although some authors [3] correlate hemangiomas with high levels of estrogen and progesterone, as it occurs with multiparity, pregnancy, and oral contraceptive use. Pathologically, they consist of interconnected endothelial-lined vascular channels, enclosed within a loose fibroblastic stroma [4]. Their vascular supply is by a hepatic arterial branch, with slow internal circulation. Hepatic hemangiomas remain stable in size, however growth is reported in some cases [5,6]. Their diagnosis is usually made during imaging examination for unrelated reasons. On CT scan they are sharply defined masses that are usually hypo-attenuating compared with the adjacent hepatic parenchyma on unenhanced images, but have a distinctive pattern of enhancement after administration of intravenous contrast medium, characterized by sequential contrast opacification usually starting at the periphery. On daily clinical practice lesions discovered incidentally on ultrasound or CT that are solitary and typical of hemangioma can be considered benign and ignored if the patient has no known or suspected primary malignancy. However, if the ultrasound or CT findings are atypical, or the patient has a known or suspected primary malignancy, an additional imaging test, either technetium-99m pertechnetate labeled red blood cell (RBC) scintigraphy or MRI, can provide a more accurate diagnosis [7]. Laboratory liver function tests are normal. The risk of rupture is minimal and does not justify resection. Other complications include thrombosis, sclerosis, and calcification. Liver hemangiomas can grow during pregnancy or with oral contraception use. Kasabach-Merritt syndrome (consumption coagulopathy) and Bornman-Terblanche-Blumgart syndrome (fever and abdominal pain) constitute uncommon complications of the lesion as one or more nodular or globular areas of enhancement, and proceeding toward the center [8,9]. Overall it should be emphasized that hepatic hemangiomas are operated only rarely and after thorough work-up, and only after a period of watchful waiting where symptoms do not subside. We reported 35 cases which were operated after extensive preoperative examinations. Actually, over this 20-year time period more than 700 cases of hepatic hemangiomas were reviewed in our hospital and only 35 cases were treated surgically, which means that < 5% of the total hepatic hemangiomas were referred for treatment and possible surgical intervention. Close observation should be reserved for asymptomatic tumors, and surgical resection is offered for symptom relief of complicated hemangiomas or lesions in which the diagnosis is uncertain [10]. When surgical intervention is required, enucleation is the preferred option for giant hemangiomas because it provides better preservation of the normal liver parenchyma compared with a formal hepatectomy [11].

Liver angiosarcoma is a rare type of liver malignancy, and traditionally is related to arsenic, vinyl chloride monomer (VCM), thorium dioxide (Thorotrast) and radium [12]. Angiosarcoma is the most common mesenchymal liver tumor and in Western countries it occurs more frequently in men with a male-to-female ratio of about 3:1 to 4:1, and most frequently in the sixth to seventh decades of life. The most common presenting symptoms are reported to be abdominal pain and non-specific systemic complaints such as weakness, fatigue, weight loss, and anorexia that appear in 25-50% of the cases [13]. Physical examination may reveal hepatomegaly, ascites, jaundice, and splenomegaly. A devastating presentation is spontaneous liver rupture and intra-abdominal bleeding, which is not uncommon (27% of the patients) and has high mortality and morbidity rates [14]. Hepatic angiosarcomas may be seen as either single or multiple masses that

are predominantly hypoattenuating compared with the surrounding hepatic parenchyma on unenhanced CT, but they may be mixed with an area of hyperattenuation caused by fresh internal hemorrhage or with areas of low attenuation near that of fluid, representing the site of old hemorrhage. Heterogeneous and progressive enhancement is showed on dynamic contrast CT scan when the tumor appears as a dominant mass [15]. Preoperative biopsy under ultrasound or CT guidance does not seem appropriate due to bleeding risk and dissemination of tumor cells among needle tract [16]. Most patients are not amenable to surgery. Usually both hepatic lobes are involved and rapid tumor growth and tendency to metastasize contribute to its dismal prognosis. Chemotherapy and radiotherapy have no role, and ligation of the hepatic artery might permit palliation. The prognosis of patients with hepatic angiosarcomas is poor with a median survival of around 6 months, and only 3% survive for more than 2 years [17].

Epithelioid hemangioendothelioma is a rare borderline tumor composed of epithelioid, endothelial and denditic cells, with an estimated prevalence of 1 per 106 people in the general population, first described by Weiss and Enzinger [18,19] and may arise in the lungs, bones, heart, brain, salivary glands and liver [20]. They have intermediate biological behavior between hemangiomas and angiosarcomas and their diagnosis is challenging although important, because long term survival may be achieved. A female to male ratio of 3:2, with a peak incidence occurring between 30 and 40 years of age is observed in a total of no more than 200 cases reported in the literature so far [21]. Clinical characteristics are non-specific and consist of right upper quadrant pain and weight loss. Physical examination may reveal hepatomegaly, a palpable mass or jaundice, and if hepatic veins invasion has occurred then Budd-Chiari syndrome is developed. Liver function tests reveal mild abnormalities in most patients and tumor marker levels are negative apart from elevated CEA levels in some patients [22]. Imaging modalities are important for prompt diagnosis. On ultrasound a hypoechoic pattern is found in the majority of the cases and on CT scan they appear as solid, non-homogeneously, hypodense nodules with a ring-like, low-density border and a low-density center. For differential diagnosis, the most important imaging features of a target sign and progressive enhancement could differentiate hepatic epithelioid hemangioendothelioma from intrahepatic multiple metastatic tumors, hemangioma and primary angiosarcoma. A definite diagnosis of the tumor can only be made by biopsy. The neoplasm is composed of epithelioid, dendritic or intermediate cells in a fibromyxoid stroma. The tumor centre becomes fibrotic, entrapping neoplastic cells. Because of its rare occurrence there is no standard treatment option. Liver transplantantion, chemotherapy or radiotherapy and liver resection have all been applied in the management of hepatic epithelioid hemangioendothelioma [19]. Five-year survival rates are better after surgical approaches and are about 75%, while no treatment has a 4.5% rate of 5-year survival. Interferon is being used as an adjuvant therapy after liver resection or transplantation and seems to reduce metastatic potential [23].

Hepatic angiomyolipoma is a benign tumor composed of variable admixtures of adipose tissue, smooth muscle, and thick-walled blood vessels, with occasional foci of extramedullary hemopoiesis. The lesion usually presents as a single, sharply demarcated but not encapsulated mass, and most of the times patients are asymptomatic. It occurs more frequently in women, with no age preference [24]. The lesion has always been regarded as benign tumor, with a slow growth and with no chances of a malignant transformation. Preoperative imaging diagnosis may be difficult, because variable proportions of fatty tissue in the tumors may result in various appearances and because of the rarity of the lesion. The tumor may be seen as an echogenic mass, either homogeneous or heterogeneous, on abdominal ultrasonography. Also, CT scan may confirm the presence of fat, but not infrequently the lesions may exhibit heterogeneous CT density. Using fat-suppression sequences or chemical shift imaging, MRI may facilitate the diagnosis. Differential diagnosis of heterogeneous fat-containing liver lesions may also include angiolipoma, adenoma, and metastatic neoplasms, such as hepatocellular carcinoma, malignant teratoma, and liposarcoma. Utility of fine needle aspiration biopsy (FNAB) is low for reaching an accurate diagnosis. General indications for the resection of angiomyolipomas are similar to other benign liver tumors and include diagnostic doubt, occurrence of symptoms, or onset of complications [25].

This study, reporting our experience in vascular liver tumors, underlines that benign vascular neoplasms should be treated only when producing symptoms and after extensive work-up to exclude other pathological processes. Also, imaging tomographic modalities can be of great help in

establishing correct diagnosis and avoid unnecessary surgical interventions. However, when diagnosis is in doubt surgical recection and thorough pathological review are the only means to deliver proper therapy in these patients.

Vascular tumors of the liver comprise an heterogeneous group of neoplasms, benign, malignant and of intermediate degree of malignancy. These lesions pose great pre– and postoperative considerations, but the correct diagnosis and histopathological classification of vascular hepatic tumors are mandatory in order to choose the correct therapeutic approach.

## References


1. Cong WM, Dong H, Tan L et al. Surgicopathological classification of hepatic space-occupying lesions:a single-center experience with literature review. World J Gastroenterol 2011; 17:2372-2378.
2. Karhunen PJ. Benign hepatic tumours and tumour like conditions in men. J Clin Pathol 1986; 39:183-188.
3. van Malenstein H, Maleux G, Monbaliu D et al. Giant liver hemangioma:the role of female sex hormones and treatment. Eur J Gastroenterol Hepatol 2011; 23:438-443.
4. Heiken JP. Distinguishing benign from malignant liver tumours. Cancer Imaging 2007; 7 (Spec No A):S1-14.
5. Mungovan JA, Cronan JJ, Vacarro J. Hepatic cavernous hemangiomas:lack of enlargement over time. Radiology 1994; 191:111-113.
6. Nghiem HV, Bogost GA, Ryan JA et al. Cavernous hemangiomas of the liver:enlargement over time. AJR Am J Roentgenol 1997; 169:137-140.
7. Vilgrain V, Boulos L, Vullierme MP et al. Imaging of atypical hemangiomas of the liver with pathologic correlation. Radiographics 2000; 20:379-397.
8. Sewell JH, Weiss K. Spontaneous rupture of hemangioma of the liver. A review of the literature and presentation of illustrative case. Arch Surg 1961; 83:729-733.
9. Corigliano N, Mercantini P, Amodio PM et al. Hemoperitoneum from a spontaneous rupture of a giant hemangioma of the liver:report of a case. Surg Today 2003; 33:459-463.
10. Duxbury MS, Garden OJ. Giant haemangioma of the liver:observation or resection? Dig Surg 2010; 27:7-11.
11. Hamaloglu E, Altun H, Ozdemir A, Ozenc A. Giant liver hemangioma:therapy by enucleation or liver resection. World J Surg 2005; 29:890-893.
12. Ho SY, Tsai CC, Tsai YC, Guo HR. Hepatic angiosarcoma presenting as hepatic rupture in a patient with long-term ingestion of arsenic. J Formos Med Assoc 2004; 103:374-379.
13. Neshiwat LF, Friedland ML, Schorr-Lesnick B et al. Hepatic angiosarcoma. Am J Med 1992; 93:219-222.
14. Locker GY, Doroshow JH, Zwelling LA, Chabner BA. The clinical features of hepatic angiosarcoma:a report of four cases and a review of the English literature. Medicine (Baltimore) 1979; 58:48-64.
15. Koyama T, Fletcher JG, Johnson CD et al. Primary hepatic angiosarcoma:findings at CT and MR imaging. Radiology 2002; 222:667-673.
16. Hertzanu Y, Peiser J, Zirkin H. Massive bleeding after fine needle aspiration of liver angiosarcoma. Gastrointest Radiol 1990; 15:43-46.
17. Falk H, Herbert JT, Edmonds L et al. Review of four cases of childhood hepatic angiosarcoma--elevated environmental arsenic exposure in one case. Cancer 1981; 47:382-391.
18. Weiss SW, Enzinger FM. Epithelioid hemangioendothelioma:a vascular tumor often mistaken for a carcinoma. Cancer 1982; 50:970-981.
19. Mehrabi A, Kashfi A, Fonouni H et al. Primary malignant hepatic epithelioid hemangioendothelioma:a comprehensive review of the literature with emphasis on the surgical therapy. Cancer 2006; 107:2108-2121.
20. Ishak KG, Sesterhenn IA, Goodman ZD et al. Epithelioid hemangioendothelioma of the liver:a clinicopathologic and follow-up study of 32 cases. Hum Pathol 1984; 15:839-852.
21. Hsieh MS, Liang PC, Kao YC, Shun CT. Hepatic epithelioid hemangioendothelioma in Taiwan:a clinicopathologic study of six cases in a single institution over a 15-year period. J Formos Med Assoc 2010; 109:219-227.
22. Lin J, Ji Y. CT and MRI diagnosis of hepatic epithelioid hemangioendothelioma. Hepatobiliary Pancreat Dis Int 2010; 9:154-158.
23. Galvao FH, Bakonyi-Neto A, Machado MA et al. Interferon alpha-2B and liver resection to treat multifocal hepatic epithelioid hemangioendothelioma:a relevant approach to avoid liver transplantation. Transplant Proc 2005; 37:4354-4358.
24. Takayama Y, Moriura S, Nagata J et al. Hepatic angiomyolipoma:radiologic and histopathologic correlation. Abdom Imaging 2002; 27:180-183.
25. Zeng JP, Dong JH, Zhang WZ et al. Hepatic angiomyolipoma:a clinical experience in diagnosis and treatment. Dig Dis Sci 2010; 55:3235-3240.