Relationships of radiological and surgical variables and vascular endothelial growth factor expression with recurrence in juvenile nasopharyngeal angiofibroma

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Abstract. Juvenile nasopharyngeal angiofibroma (JNA) is a rare fibrovascular tumor that is histologically benign but clinically invasive and it is typified by a high recurrence rate. The recurrence rate is influenced by several characteristics including sociodemographic, clinical, radiological, and surgical variables and vascular endothelial growth factor (VEGF) expression. This observational retrospective cohort study aimed to clarify the relationships of these characteristics with the risk of recurrence in 38 male patients with JNA, including 11 patients with recurrence. JNA commonly develops in the second decade of life with a mean age of occurrence of 15.8 years. Nasal blockage and epistaxis were the most common complaints. Young age, early onset, late-stage disease, and high VEGF expression were linked to higher recurrence rates, albeit without significance. Meanwhile, the age of onset, the presence of an oropharyngeal mass, tumor stage, the use of preoperative embolization, and the intensity of VEGF staining appeared to have clinical significance regarding the risk of recurrence. In this study, preoperative embolization did not decrease the recurrence rate. The risk of recurrence was increased by the presence of residual tumor, suggesting that measures should be taken to ensure complete tumor resection during surgery.

1. Introduction
Juvenile nasopharyngeal angiofibroma (JNA) is a rare vascular tumor that is histologically benign but clinically malignant. It is often associated with high rates of morbidity, mortality, and recurrence. Patients with JNA, in general, present with one-sided nasal congestion, usually accompanied by recurrent and severe epistaxis that is difficult to treat with standard therapies. JNA comprises 0.05% of all head and neck tumors, with an incidence of 1 in every 5000–60,000 ear, nose, and throat patients [1-4].

According to Glad et al. [5], nasal congestion and epistaxis are the most common symptoms in patients with JNA, with a prevalence of 91% and 63%, respectively. Other accompanying symptoms include nasal secretions, pain, sinusitis, facial deformity, hearing loss, otitis media, proptosis, and diplopia. Symptoms generally appear 6 months to 1 year before diagnosis in males aged 10–24 years (median age at diagnosis, 15 years) [4,5].

The causes of JNA are unknown; however, it is suspected to be associated with sex hormones. Tumors typically appear in adolescent boys, and lesions generally regress after the complete development of secondary sex characteristics, providing evidence of hormonal influences on
tumor growth. Angiogenesis and hormonal factors are being explored for research on JNA, although the available data are insufficient for definitive conclusions and the mechanisms remain unclear. Anggreani et al. [6] reported histochemical examination results illustrating ER-β expression in all patients with JNA. Saylam et al. [7] found that estrogen and progesterone did not play important roles in the occurrence of angiogenesis in JNA. Schuon et al. [8] obtained data supporting the concept that growth and vascularization in JNA are influenced by factors released by fibroblasts in stroma. These findings support the hypothesis that JNA has similar biological characteristics as angiogenic tumors. Beham et al. [9] stated that the stromal cell type in JNA is fibroblasts and not myofibroblasts. Wendler et al. [10] reported that in addition to the vascular and stromal fibrous components, the presence of inflammatory cells such as mast cells and lymphocytes should also be considered in this tumor. Brieger et al. [11] revealed that vascular endothelial growth factor (VEGF) was expressed by stromal and endothelial cells, resulting in increased vessel density in JNA [7-13].

The clinical features of JNA depend on the tumor location and the stage of the disease at diagnosis. The tumor spreads via the submucosal and soft tissues around the lesion. The tumor may spread anteriorly into the nasal cavity, superiorly toward the sphenoid sinuses and sella, or laterally through the sphenopalatine foramen toward the pterygomaxillary fossa, infratemporal fossa, and infraorbital fissure. The tumor may also spread to the intracranial space through the sella or to the fossa cranium media through the laser foramen. Sennes et al. [12] reported that JNA mostly invades the superior and lateral regions and that expansion toward the pterygopalatine fossa results in increased morbidity.

JNA is classified on the basis of tumor spread. This classification is useful for devising a management plan, and thus, a universal classification system is needed. The primary management of JNA is surgery, which can be performed via endoscopy or an external approach. JNA is an extremely aggressive tumor with a strong tendency to bleed; therefore, preoperative management to reduce blood flow to the tumor via embolization is required. An alternative to embolization is carotid artery ligation as some hospitals may lack the facilities and infrastructure required for embolization. Gupta et al. [13] concluded that surgical techniques should be based on the tumor location and should be selected primarily for younger patients and that surgery should aim to minimize facial growth malformation. Nicolai et al. [14] noted that nasoendoscopy is not recommended for lesions involving the internal carotid artery, cavernous sinus, or optic nerves. Wormald et al. [15] stated that endoscopic surgery is effective for JNAs that have extended to the pterygopalatine fossa and slightly to the infratemporal fossa [14-17]. In addition to the management of JNA, recurrence rate is also an important factor. Sun et al. [16] obtained a recurrence rate of 48.2% for patients younger than 18 years. Meanwhile, Herman et al. [17] and Tyagi et al. [18] reported recurrence rates of 27.5% and 50%, respectively. On the other hand, no data describing the characteristics of JNA and their association with recurrence have been reported in Indonesia.

This study aimed to determine the relationship of the sociodemographic, clinical, radiological, surgical, and histopathological characteristics and VEGF expression with JNA recurrence in an effort to predict the recurrence risk.

2. Materials and Methods
This was an observational study with a retrospective cohort approach. The study protocol was approved by Health Research Ethics Committee, Faculty of Medicine Universitas Indonesia-Cipto Mangunkusumo Hospital. Patients with JNA, based on the medical records, who underwent surgery between July 2010 and July 2011 were included. Screening was performed according to the completeness of medical records and radiological data and the quality of paraffin blocks. Data obtained from medical records included sociodemographic (age and sex), clinical (anamnesis regarding onset and physical examination data), and surgical characteristics (surgical technique and amount of bleeding during surgery). From the obtained radiological data, the tumor size, tumor stage, number of feeding vessels, and use of presurgical embolization were reassessed. Histopathological data were obtained after immunohistochemical staining with VEGF as the primary antibody. The
intensity of VEGF staining and the number of VEGF-positive tumor cells in the stroma and endothelium were assessed.

All collected data were edited and coded, after which they were entered into a worksheet and processed using SPSS 20 software. The collected data were descriptively analyzed with emphasis on the type of data scale. Numerical data are presented as the mean and standard deviation, and categorical data are presented as frequencies (n) and percentages (%). Differences in proportions of >10% indicated a significant clinical difference. Bivariate analysis was used to examine the relationship between independent and dependent variables. For variables on an ordinal/dichotomous scale, analysis was performed using the chi-squared test. Differences were considered significant at p < 0.05. To view and predict the effects of the independent variables on the dependent variables, logistic regression analysis was used.

### 3. Results

Of 71 recorded cases, complete medical records, complete radiological data, and good paraffin blocks were available for 49, 42, and 38 cases, respectively. Among the 38 patients included in the analysis, 11 experienced recurrence. Data on the distribution of sociodemographic, clinical, radiological, and surgical characteristics and VEGF expression are presented in Table 1.

#### Table 1. Distribution of sociodemographic characteristics among patients with juvenile nasopharyngeal angiofibroma (n = 38)

| Variable | Recurrence | No recurrence | Total |
|----------|------------|---------------|-------|
| **Age** |            |               |       |
| ≤18 years old | 10          | 23            | 33    |
| >18 years old | 1           | 4             | 5     |
| **Gender** |            |               |       |
| Male | 11          | 27            | 38    |
| Female | 0           | 0             | 38    |

The age range of the included patients was 12–30 years, with most patients being younger than 18 years. The clinical characteristics of the study participants are listed in Table 2.

#### Table 2. Distribution of clinical characteristics (n = 38)

| Variables | Recurrence | No recurrence | Total |
|-----------|------------|---------------|-------|
| **Symptoms onset (before diagnosis)** |            |               |       |
| ≤6 months | 7          | 12            | 19    |
| >6 months | 4          | 15            | 19    |
| **Clinical symptom** |            |               |       |
| Nasal congestion | 11          | 27            | 38    |
| Epistaxis | 11          | 27            | 38    |
| Rhinorrhea | 4           | 3             | 7     |
| Interruption of smell | 4           | 10            | 14    |
| Hearing disorders | 6           | 7             | 13    |
| **Nasal cavity mass** |            |               |       |
| Bilateral | 7          | 17            | 24    |
| Unilateral | 4           | 10            | 14    |
| **Throat mass** |            |               |       |
| Yes | 1           | 8             | 9     |
| No | 10          | 19            | 29    |
Epistaxis and nasal congestion were the most common complaints (100%), whereas rhinorrhea was the least frequent complaint (7 cases). Physical examination of the nose revealed unilateral and bilateral nasal cavity masses in 14 and 24 patients, respectively. Of the 14 patients with unilateral nasal cavity masses, 4 (28%) experienced recurrence compared with 7 of the 24 patients (29%) with bilateral nasal cavity masses. Additionally, throat masses were identified in 9 patients (23.4%).

Radiological characteristics including tumor size, tumor stage, number of feeding vessels, and use of presurgical embolization were assessed as listed in Table 3.

**Table 3. Distribution of radiological characteristics**

| Variable               | Recurrence | No recurrence | Total |
|------------------------|------------|---------------|-------|
| **Tumor size**         |            |               |       |
| >4 cm                  | 8          | 20            | 28    |
| ≤4 cm                  | 3          | 7             | 10    |
| **Tumor stage**        |            |               |       |
| Late (stage III)       | 10         | 19            | 29    |
| Early (stages I and II)| 1          | 8             | 9     |
| **Feeding vessel**     |            |               |       |
| Internal carotid artery| 4          | 3             | 7     |
| External carotid artery| 7          | 13            | 20    |
| **Embolization**       |            |               |       |
| Yes                    | 8          | 13            | 17    |
| No                     | 3          | 14            | 21    |

The feeding vessel was identified only in 27 patients as some patients did not undergo angiography. Among 20 patients for whom the external carotid artery was the feeding artery, 7 experienced recurrence. Of 28 patients with tumors of at least 4 cm in diameter, 8 experienced recurrence. Twenty-nine patients had late-stage disease, 10 of whom exhibited recurrence. In total, 21 patients underwent embolization prior to surgery, whereas 17 patients underwent carotid artery ligation.

The assessed surgical characteristics, namely surgical technique and the amount of bleeding, are presented in Table 4.

**Table 4. Distribution of surgical characteristics**

| Variable          | Recurrence | No recurrence | Total |
|-------------------|------------|---------------|-------|
| **Surgical technique** |            |               |       |
| Endoscopy         | 4          | 7             | 11    |
| External          | 7          | 20            | 27    |
| **Bleeding**      |            |               |       |
| >1000 mL          | 4          | 12            | 16    |
| ≤1000 mL          | 7          | 15            | 22    |

All included patients were treated surgically. In total, 11 patients (28.9%) were treated via an endoscopic approach, whereas 24 (63.2%) and 3 patients (7.9%) patients were treated via transpalatal and dual approach (combination of the Weber–Ferguson approach and transpalatal surgery), respectively. Meanwhile, of the 27 patients who underwent surgery via the external approach, 7 experienced recurrence.

The assessed histopathological characteristics were the intensity of VEGF staining and percentage of VEGF-positive tumor cells (Table 5). Overall, 5 of 10 patients (50%) with high intensity of VEGF staining experienced recurrence, whereas all patients had a high percentage of VEGF-positive tumor cells.
Table 5. Distribution of histopathological characteristics

| Variable                        | Recurrence | No recurrence | Total |
|---------------------------------|------------|---------------|-------|
| **Intensity of VEGF staining**  |            |               |       |
| Strong                          | 5          | 5             | 10    |
| Weak                            | 6          | 22            | 28    |
| **Percentage of VEGF-positive cells** |      |               |       |
| High                            | 11         | 27            | 38    |
| Low                             | 0          | 0             | 0     |

VEGF, vascular endothelial growth factor

Table 6 illustrates the relationships of the sociodemographic, clinical, radiological, and surgical characteristics and VEGF expression with recurrence. According to Fisher’s absolute test, none of the assessed risk factors was associated with recurrence (all $p > 0.05$).

Table 6. Relationships of various patient characteristics with recurrence

| Risk factor                    | $p$   |
|--------------------------------|-------|
| Age                            | 0.545 |
| Onset                          | 0.238 |
| Nasal cavity mass              | 0.634 |
| Throat mass                    | 0.179 |
| Tumor size                     | 0.615 |
| Tumor stage                    | 0.179 |
| Feeding vessel                 | 0.279 |
| Embolization                   | 0.153 |
| Surgical technique             | 0.393 |
| Bleeding                       | 0.466 |
| Intensity of VEGF staining     | 0.098 |

Note: Based on Fisher’s absolute test. VEGF, vascular endothelial growth factor

Conversely, onset, throat mass, staging, and intensity of VEGF staining revealed a $p$ value of <0.25; thus, these may be predictive of recurrence. Binary logistic regression was further performed to determine the relative risk of recurrence associated with each variable, as presented in Table 7.

Table 7. Results of bivariate analysis

|                      | Recurrence | No recurrence | $p$   | RR   | 95% CI Min. | Max. |
|----------------------|------------|---------------|-------|------|-------------|------|
| Onset                | ≤6 months  | 7             | 12    | 0.24 | 1.75        | 0.61 | 5.00 |
|                      | >6 months  | 4             | 15    |      |             |      |      |
| Throat mass          | Present    | 1             | 8     | 0.18 | 0.322       | 0.05 | 2.18 |
|                      | Absent     | 10            | 19    |      |             |      |      |
| Stage                | Late       | 10            | 19    | 0.18 | 3.10        | 0.05 | 2.18 |
|                      | Early      | 1             | 8     |      |             |      |      |
| Embolization         | Yes        | 8             | 13    | 0.15 | 2.159       | 0.67 | 6.90 |
|                      | No         | 3             | 14    |      |             |      |      |
| Intensity of VEGF staining | Strong | 5             | 5     | 0.09 | 2.3         | 0.16 | 1.09 |
|                      | Weak       | 6             | 22    |      |             |      |      |

RR, relative risk; CI, confidence interval
The results illustrated that none of the variables was significantly linked to recurrence risk. To determine the effects of the combinations of these five variables on recurrence, a multivariate logistic regression model was used, as listed in Table 8.

Table 8. Results of multivariate logistic regression analysis

|            | Coeff. | SE  | Wald | df | p    | OR    | 95% CI Min. | Max. |
|------------|--------|-----|------|----|------|--------|--------------|------|
| Onset      | 0.292  | 0.87| 0.111| 1  | 0.739| 1.339  | 0.241        | 7.453|
| Throat mass| −0.892 | 1.25| 0.508| 1  | 0.476| 0.274  | 0.035        | 4.766|
| Embolization| 0.837  | 0.93| 0.813| 1  | 0.367| 2.309  | 0.374        | 14.252|
| Stage      | −1.636 | 1.24| 1.746| 1  | 0.186| 0.163  | 0.017        | 2.205|
| Staining   | −1.611 | 0.96| 2.829| 1  | 0.093| 0.272  | 0.031        | 1.305|
| Intensity  | −0.43  | 0.97| 0.022| 1  | 0.883| 0.866  |              |      |

OR, odds ratio; CI, confidence interval;

The variables onset and embolization both revealed odds ratios of >1, indicating that they increased the recurrence risk in combination.

4. Discussion

Of the 71 total patients identified between July 2010 and June 2011, only 42 patients had complete medical records and radiology but 4 cases cannot find the paraffin blocks. Recurrence was reported for 28 patients. Thus, the recurrence rate in the entire cohort did not differ significantly from that among the 38 patients who met the inclusion criteria for our analysis.

The age range of the patients in this study did not differ significantly from the previous studies. Panda et al. [2] reported an age range of 9–27 years for 150 patients with JNA in India. Similarly, Carrillo et al. [20] reported an age range of 18–35 years and an average age of 18.5 years among 54 patients in Mexico. In a study in Denmark, Glad et al. [5] included 51 patients with an age range of 10–24 years (median, 15 years). The age range and median age in a study of 41 patients with JNA by Dharmabakti were 10–29 and 17.3 years, respectively. Similarly, a 27-patient cohort reported by Anggreani et al. indicated an age range of 9–23 years and an average age of 15.7 years [2,6,19,20].

In accordance with our findings, Radkowski et al. found that nasal obstruction was the most frequent complaint among patients with JNA (87%), followed by epistaxis (82%). Meanwhile, facial deformity and proptosis were rare, being identified in only 8.7% and 4.35% of patients, respectively. Carrillo et al. observed nasal congestion and epistaxis in 76% and 15% of patients, respectively. Panda et al. detected nasal obstruction and epistaxis in all enrolled subjects, whereas only 4 of 150 patients experienced optalmophlegia. In a study of 41 patients with JNA, Dharmabakti et al. reported that nasal obstruction was the major complaint (89.2%), followed by epistaxis (83.8%) chronic rhinorrhea (48.7%), headache (24.3%), rhinolalia (10.8%), and anosmia (5.4%) [2,19,20].

Computed tomographic angiography was performed in some patients in this study, which allowed the identification of feeding vessels prior to surgery. In a prior research, Carrillo et al. [20] performed embolization in 54 patients before surgery. Llorente et al. [21] performed angiography and preoperative embolization in 39 patients (91%) and observed bleeding from the external carotid artery, internal carotid artery, and both arteries in 34, 1, and 8 patients, respectively.

The overall amount of blood loss in this study was 100–12,000 ml (mean, 600 ml). Meanwhile, among patients for whom the feeding vessel was identified but not embolized, the amount of blood
loss was 600–12,000 (mean, 3100 ml). Thus, it appears that embolization can reduce intraoperative bleeding [20,22].

Our findings that all patients exhibited VEGF positivity are in accordance with the research conducted by Saylam et al. [7] involving immunohistochemical examination of estrogen receptor (ER), progesterone receptor (PR), proliferating cell nuclear antigen (PCNA), VEGF, and transforming growth factor β (TGF-β) expression using anatomical pathology preparations. Of 27 samples, only 2 were positive for ER, whereas 9, 27, 24, and 14 samples were positive for PR, PCNA, VEGF, and TGF-β, respectively. The data indicated that estrogen and progesterone were not crucial in the occurrence of angiogenesis in JNA. PCNA, VEGF, and TGF-β are assumed to enhance proliferation and angiogenesis [7]. Although statistical significance was not observed in our study, the numerical difference in the percentages of the samples with strong and weak VEGF expression as well as the relative risk between the expression categories suggests that the extent of VEGF expression has biological importance. It is possible that statistical significance would be observed if a larger sample size is used; however, additional research is needed to confirm this hypothesis.

Meanwhile, the research conducted by Lloyd et al. [23] indicated that embolization can obscure the borders of tumors, especially those that invade the sphenoid bone, increasing the likelihood that the entire tumor will not be excised during surgery. The presence of tumor residue permits JNA recurrence. This is in contrast to the results of a study conducted by Gailard et al. [24], who concluded that recurrence was associated with tumor stage and the absence of presurgical embolization.

5. Conclusion
The incidence of JNA was the highest in the second decade of life, with a mean patient age of 15.8 years at the time of diagnosis. Recurrence appeared to be associated with younger age, rapid onset, late-stage disease, and high intensity of VEGF staining. In contrast, the sociodemographic, clinical, radiological, and surgical variables and the intensity of VEGF staining were not significantly associated with the risk of recurrence, and presurgical embolization did not reduce recurrence rates.

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