# Neutrophil hypersegmentation in children receiving angiotensin converting enzyme inhibitors

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**Captopril and enalapril are the most commonly used angiotensin converting enzyme inhibitors in several cardiac diseases in children. On the other hand, the intrinsic renin-angiotensin system in the bone marrow might affect the growth of hematopoietic colonies and cellular production, proliferation and differentiation in physiological and pathological states. Starting with the hypothesis that inhibition of the renin-angiotensin system may have some effects on the hematopoietic system, including morphological changes within the granulocytes, we thus aimed to investigate prospectively whether the use of angiotensin converting enzyme inhibitors has any effect on the morphology, and especially segmentation, of neutrophils in peripheral blood. A total of 40 children with various heart diseases receiving either of two angiotensin converting enzyme inhibitors (captopril or enalapril) aged between 2 to 16 years were enrolled, and 40 healthy age- and sex-matched children were enrolled as controls. Complete blood count, peripheral blood smear, liver and renal function tests, and measurement of serum alkaline phosphatase, ferritin, vitamin $B_{12}$ and folate levels were performed in all cases. Peripheral blood smears were viewed by two pediatric hematologists in a blinded manner. Neutrophil hypersegmentation was described as presence of five or more neutrophils with five well-separated lobes or at least one neutrophil with six or more lobes among 100 segmented neutrophils. The number of patients with neutrophil hypersegmentation in the study group was significantly higher than in the control group, and the mean lobe count in the study group was significantly higher than in the control group. Neutrophil hypersegmentation, as detected in patients using angiotensin converting enzyme inhibitors in the present study, has not been reported previously. Further studies aiming to explain the pathophysiological mechanism(s) underlying neutrophil hypersegmentation in patients receiving angiotensin converting enzyme inhibitors are needed.**

***Key words: neutrophil hypersegmentation, children, angiotensin converting enzyme inhibitors, captopril, enalapril.***

Angiotensin converting enzyme (ACE) inhibitors lower aortic pressure and systemic vascular resistance, do not affect pulmonary vascular resistance significantly, and lower left atrial and right atrial pressures in heart failure. All these effects decrease left-to-right shunt, mitral regurgitation (MR) and aortic regurgitation (AR), and induce ejection fraction, fractional shortening and systemic blood flow in children with left ventricular dysfunction. Therapeutic trials of ACE inhibitors have been reported in children with left-to-right shunt, AR and MR, and Fontan circulation. Captopril and enalapril are the two ACE inhibitors most commonly used in such cardiac indications in children[1].

Several side effects including angioedema, rash, taste disturbances and cough associated with the use of ACE inhibitors have been reported[2]. Anemia, granulocytopenia, pancytopenia and aplastic anemia are hematological side effects attributed to the use of ACE inhibitors[3,4].

Accumulating evidence indicates that the local renin-angiotensin system (RAS) in the bone marrow may be operative in normal and pathological hematopoiesis[5]. Intrinsic RAS in the bone marrow might affect the growth of hematopoietic colonies and cellular production, proliferation, and differentiation in physiological and pathological states. It seems likely that inhibition of RAS may have some effects on the hematopoietic system, including morphological changes within the granulocytes. Thus, we aimed to investigate in a prospective manner whether the use of ACE inhibitors has an effect on the morphology, and especially segmentation, of neutrophils in peripheral blood.

## Material and Methods

This study was performed in the Department of Pediatrics of Gülhane Military Medical Academy between March 2004 and March 2006. A total of 40 children with various heart diseases receiving either of two ACE inhibitors (captopril or enalapril) aged between 2 to 16 years were enrolled. Forty healthy age- and sex-matched children were enrolled as controls. Patients were excluded if they had a concomitant disease such as renal failure, liver disease or nutritional anemia, or if they were receiving different drugs other than ACE inhibitors. Complete blood count (CBC), peripheral blood smear, liver and kidney function tests, and measurement of serum alkaline phosphatase (ALP), ferritin, vitamin $B_{12}$ and folate levels were performed in all cases. Peripheral blood smears were viewed by two pediatric hematologists who were blinded to both the laboratory results and to group assignments of the smears. Three hundred neutrophils from each case were examined. Only lobes connected by a fine thread of chromatin were counted. A total of 12,000 cells were viewed for each group. Neutrophil hypersegmentation (NH) was described as presence of five or more neutrophils with five well-separated lobes or at least one neutrophil with six or more lobes among 100 segmented neutrophils[6].

Statistical comparison between the groups was performed by using SPSS for Windows 10.0. Mann-Whitney U test was used for non-parametric data. A p value less than 0.05 was considered significant.

## Results

The demographic characteristics of the patients and control subjects are shown in Table I. Complete blood count, ALP, liver function tests, and kidney function tests were within reference ranges according to their ages, and serum ferritin, vitamin $B_{12}$ and folate levels were also within normal limits in both the patient and control groups[7] (Table I). No statistically

**Table I.** Demographic Characteristics of the Patients Receiving Angiotensin Converting Enzyme Inhibitors and Controls

| | Patients (n=40) | Controls (n=40) |
|---|---|---|
| Age (years)* | 9.0 (2-16) | 9.0 (3-16) |
| Gender (F/M) | 20/20 | 16/20 |
| Diagnosis | | |
| Aortic regurgitation (AR) | 14 | |
| Mitral regurgitation (MR) | 16 | |
| AR+MR | 4 | |
| Congenital heart disease with hemodynamically significant left- to-right shunt | 6 | |
| Duration of treatment (months)* | 48.0 (2-144) | |
| Drug doses (mg/kg/d)* | | |
| Captopril | 1.0 (0.5-1.8) | |
| Enalapril | 0.12 (0.07-0.25) | |

*: Values in parentheses indicate the mean range.

significant differences were found between age, sex, CBC, and biochemical tests of the study and control groups (p>0.05) (Table II).

Results of the NH are given in Table III. The number of patients with NH was greater (5/40 vs 0/40, p<0.0001) and the mean lobe count was significantly higher (2.71 vs 2.17, p<0.001) in the study group when compared to the control group.

**Table III.** Comparison of the Number of Cases with Neutrophil Hypersegmentation and of the Mean Lobe Count in the Study and Control Groups

| | Study group (n=40) | Control group (n=40) | p value |
|---|---|---|---|
| Neutrophil hypersegmentation n (%) | 5 (12.5) | 0 (0) | <0.0001 |
| Mean lobe count | 2.71 | 2.17 | <0.001 |

In an attempt to determine the effect of the duration of the treatment on NH, we investigated the correlation between the duration of treatment and the number of neutrophil lobes, and treatment duration mildly but significantly correlated with neutrophil lobe counts (r=0.396, p<0.05). We made a further analysis to determine if the use of either captopril or enalapril had different effects on hypersegmentation. There were no statistically significant differences between the data of captopril and enalapril. However, there was a significant positive correlation between the neutrophil lobe counts and the duration of captopril treatment (r=0.674, p=0.003). When we investigated the relationship between the (type of) specific cardiac diagnoses and NH, there were no significant correlations between the type of cardiac pathology and NH, since 2, 2 and 1 of the patients with NH had aortic regurgitation, mitral regurgitation and congenital heart disease with hemodynamically significant left-to-right shunt, respectively.

## Discussion

Neutrophil hypersegmentation is a characteristic feature of disturbed hematopoiesis in conditions such as megaloblastic anemia, iron deficiency anemia (IDA), use of various drugs (e.g. steroids, granulocyte colony stimulating factor [G-CSF], recombinant human interleukin-2 [rhIL-2], antithymocyte globulin [ATG]),

**Table II.** Hematological Profile of the Study and Control Groups

| | Hb (g/dl) | RBC ($x10^6$/µl) | MCV (fl) | MCH (pg) | RDW (%) | WBC ($x10^3$/µl) | Plt ($x10^3$/µl) | Ferritin (ng/ml) | $B_{12}$ (pg/ml) | Folate (ng/ml) |
|---|---|---|---|---|---|---|---|---|---|---|
| Study group | | | | | | | | | | |
| Median | 12.6 | 4.9 | 80.4 | 26.7 | 13.3 | 7.6 | 323 | 84.2 | 312.2 | 4.7 |
| Range | 11.3-13.8 | 3.7-5.2 | 78-92.3 | 24-32.1 | 11.2-15.1 | 3.4-17.8 | 231-619 | 24.1-128.6 | 154.4-582.1 | 2.1-8.3 |
| Control group | | | | | | | | | | |
| Median | 13.2 | 4.7 | 79.6 | 27.3 | 14.6 | 7.1 | 297 | 79.6 | 319.6 | 5.1 |
| Range | 11.2-16.6 | 3.9-5.3 | 72.2-89 | 23.6-31 | 10.3-15.9 | 3.2-19.1 | 193-728 | 18.3-117.4 | 170.4-640.1 | 1.9-8.7 |

Hb: Hemoglobin. RBC: Red blood cells. MCV: Mean corpuscular volume. MCH: Mean corpuscular hemoglobin. RDW: Red cell distribution width. WBC: White blood cells. Plt: Platelets.

kidney diseases, myelokathexis, malignancies, heat stroke and radiation exposure[6,8-18]. We thus investigated whether the use of ACE inhibitors caused NH since ACE inhibitors may affect hematopoiesis via local RAS in the bone marrow.

Cell proliferation, differentiation, immigration to peripheral blood and life span of the cell in the bone marrow are determined by cytokines, growth factors, specific cell surface receptors and the inductive role of the local microenvironmental signals in the bone marrow. RAS is also a system that has paracrine-autocrine effects on many tissues and organs. Angiotensin II is the dominant-effective peptide of RAS, and regulates cellular growth in tissues. RAS in the bone marrow has effects on the growth of hematopoietic colonies and cellular production, proliferation and differentiation[5].

RAS not only affects erythropoietic progenitors but also has effects on pluripotent hematopoietic stem cell colonies[19]. Plasma concentrations of "tetrapeptide N-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP)", a negative regulator of normal hematopoietic stem cells, are determined physiologically by ACE. ACE, with its N-active and C-active areas, demonstrates hydrolyzing dipeptidyl activity against domain AcSDKP. N-active terminal hydrolyzes AcSDKP 50 times more rapidly in comparison to C-active terminal. ACE contributes to the transition of primitive stem cells in the hemapoietic bone marrow to S phase hydrolyzing AcSDKP[20]. AcSDKP decreases prominently with increasing ACE content of stromal cells in the bone marrow microenvironment. AcSDKP escaping from the hydrolysis of ACE during captopril use takes hematopoietic stem cells out of cyclus[19]. Azizi et al.[21] demonstrated 90 to 99% inhibition in AcSDKP hydrolysis and 5.5 times increase in plasma AcSDKP levels after one dose administration of captopril to healthy volunteers. Two- to 5- times increases in plasma and urine concentrations of AcSDKP have been demonstrated with the use of enalapril[22]. A prominent effect of ACE inhibitor on hematopoietic progenitors in healthy individuals has been demonstrated in this study. Colony forming units of granulocyte-monocyte (CFU-GM) and burst-forming units of erythroid (BFU-E) decreased and mixed colony forming unit (CFU-mixed) increased prominently with the use of enalapril.

The relationship between the RAS and hematopoietic system is multidimensional. Several types of blood cells have been demonstrated to carry RAS elements. Renin expression in macrophages and monocytes of rats[23], and angiotensinogen synthesis in leukocytes of rats[24] have been demonstrated. Cathepsin G, one of the non-renin enzymes forming angiotensin from angiotensinogen, is present in human leukocytes[25], and cathepsin G and D are present in bone marrow[26]. AT1 receptors are present in human thrombocytes[27]. The finding of NH without any findings of cytopenia in patients using ACE inhibitors in our study may be the result of this relationship between RAS and hematopoiesis. The inhibition of RAS, which has important roles in each stage of cell production, proliferation and differentiation, may explain the etiology of NH in our study group.

Neutrophil hypersegmentation usually results from the deficiency or utilization disorder of vitamin $B_{12}$ or folate. Decrease in DNA synthesis is the mechanism responsible for morphological changes observed in megaloblastosis[9]. We did not observe any deficiency of vitamin $B_{12}$ or folate in any of our cases in the study group.

Apart from the deficiency of vitamin $B_{12}$ and folate, NH has also been reported in several pathological states affecting bone marrow directly or indirectly. The association of IDA and NH has been emphasized in many case reports and studies[8,9,28]. Although it is not exactly clear why NH is observed in IDA, it has been hypothesized that iron might be effective in the metabolism of folate and in the synthesis of granulocyte DNA[28]. Severe iron deficiency may disrupt the utilization of vitamin $B_{12}$ and folate at the cellular level[29], and intraerythrocytic folate levels increase after iron treatment in patients with IDA[30].

The etiology of NH observed with the use of drugs such as G-CSF, rhIL-2 and ATG has not been clarified exactly[10-13].

Myelokathexis is a cause of severe chronic neutropenia characterized by degenerative changes and hypersegmentation in mature neutrophils. A long-time retention of neutrophils in the bone marrow compartment has been deemed responsible[14].

Neutrophil hypersegmentation has been reported in patients with malignant lymphoma[15], hyperthermia and heat stroke[16,17], and during radiotherapy[18], although the physiopathological mechanisms are not exactly clear.

The hematological side effects and adverse effects of ACE inhibitors have been well known for a long time. Blockage of the poietic effects of RAS on bone marrow might have negative qualitative and quantitative effects on all blood cells. The NH detected in patients using ACE inhibitors in the present study has not been reported previously. Further studies aiming to explain the pathophysiological mechanism(s) underlying NH in patients receiving ACE inhibitors are needed.

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