*Original Research*

# Mean Corpuscular Hemoglobin Concentration in Hemoglobin CC, SC, and AC

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**Point mutation in the hemoglobin beta chain generates many deviants from the normal format hemoglobin A, which contains 2 alpha chains and 2 beta chains. Among the numerous deviants, Hemoglobin C (HbC) and Hemoglobin S (HbS, sickle cell hemoglobin) are 2 of the most common variants with clinical significance. Mean corpuscular hemoglobin concentration (MCHC) is of a measurement of the concentration of hemoglobin in a given volume of packed red blood cells. It is one of the red cell indices measured daily in every blood specimen, which provides clues to the clinicians about the quality of the red cells and the associated diseases. Previous studies have suggested that patients with HbCC and HbSC, and up to 45% patients with HbAC present with high MCHC. However, in our practice we found that the MCHC values in these patients showed a broad spectrum. To clarify this discrepancy, 388 peripheral blood specimens with HbCC (homozygous C), HbSC (compound heterozygous S and C), or HbAC (heterozygous C) were pulled out from hospital's medical records. We found about 50% of the HbCC patients and 20% of HbSC patients had elevated MCHC, and in HbAC patients, elevation of MCHC was rare. Therefore, our study sheds new light on the interpretation of MCHC in daily practice.**
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## INTRODUCTION

Hemoglobin C is resulted from a point mutation in beta chain, in which the glutamate on position 6 is replaced by lysine.[1] Homozygous HbC (HbCC) exhibits a stronger net intermolecular attraction and subsequently forms intra-erythrocytic crystals when hemoglobin is oxygenated.[2,3] Moreover, studies showed that HbCC erythrocytes contain higher levels of membrane-associated hemichromes and more extensively clustered band 3 protein, which partially contribute to the rigidity of the red cells.[4] These, together with some other mechanisms, such as loss of intracellular potassium, dehydration,[5] etc., are responsible for the aberrant physical properties of the erythrocytes and the related clinical consequences. HbC can compound with other hemoglobin mutants to form compound heterozygotes. The commonly one is HbSC, containing equal levels of HbS and HbC. HbSC exhibits a moderately severe phenotype in spite of being a mixture of HbS trait and HbC trait, neither of which has significant pathology. The mechanism behind this is not quite clear, possibly due to that HbC dehydrates the HbSC red cells and exacerbates the sickling propensity.[6-8]

Mean corpuscular hemoglobin concentration (MCHC) is of a measurement of the concentration of hemoglobin in a given volume of packed red blood cells. It is traditionally calculated by dividing the hemoglobin quantity by the hematocrit. MCHC is reduced (hypochromic) in some types of the microcytic anemia, in which the degree of loss of hemoglobin is more than loss of cell volume, but normal (normochromic) in macrocytic anemia, in which hemoglobin is increased, but the cell size is also proportionally increased. MCHC is elevated (hyperchromic) in hereditary spherocytosis and sickle cell disease.[9]

Many studies in the literature suggest that HbCC and HbSC have universally elevated MCHC, and up to 45% of HbC trait (HbAC) have significantly increased MCHC value also.[7,10-12] The explanation lies in the fact that the rigidity and dehydration of these abnormal hemoglobins causes microcytosis and compacted hemoglobins. Some researchers go further to use MCHC to differentiate HbC from other hemoglobinopathies, such as HbE trait.[10,11]

However, we find from time to time that the MCHC in portion of HbCC and HbSC cases are within normal reference range, which is contradictory to what are described in textbooks and literature. To clarify this discrepancy, we retrieved certain amount cases of HbCC, HbSC, and HbAC to conduct a data analysis. The quality controls of the hemoanalyzer have also been reviewed.

## MATERIALS AND METHODS

The HPLC results and the correspondent complete blood counts (CBC) of 78 cases of HbCC, 243 cases of HbSC, and

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67 cases of HbAC were retrieved from medical records. The various hemoglobin fractions (percentage) and CBC parameters were analyzed and compared. One month of quality controls for the hemoanalyzer (Simens Advia 2120) were also reviewed. All the specimens were de-identified. The entire study process and the related results are for research only and will not affect patients' diagnoses or healthcare management.

**Table 1.** The hematological parameters and hemoglobin fractions in patients with HbCC, HbSC, or HbAC.

| | case | age | RBC | Hb | MCV | MCH | MCHC | RDW | F | P3 | A0 | A2 | C | S |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HbCC | 78 | 37.9 | 4.7±1.6 | 11.4±3.2 | 68.7±17.6 | 24.3±6.4 | 35.4±3.4 | 17.9±5.6 | 7.9±30.4 | 0.19±3.8 | 1.0±3.8 | 3.5±2.4 | 84.2±26.6 | 3.3±3.8 |
| HbSC | 243 | 30.1 | 4.1±1.5 | 10.9±3.3 | 77.5±15.8 | 27.0±4.9 | 34.8±2.9 | 17.3±4.5 | 2.0±7.8 | 0.10±2.7 | 1.5±5.6 | 4.1±1.3 | 45.5±3.2 | 47.0±3.6 |
| HbAC | 67 | 17.5 | 4.4±1.2 | 11.8±2.8 | 77.5±13.6 | 26.4±5.2 | 34.3±1.8 | 14.9±2.8 | 1.4±6.0 | 2.4±1.0 | 55±8.4 | 3.3±0.6 | 34.2±6.4 | 1.3±0.6 |
| Ref. Range | | | 4.2-5.4 | 12.0-16.0 | 71.4-94.6 | 26.6-31.6 | 30.5-35.5 | | | | | | | |

RBC: red blood cells, unit- million/uL; Hb: hemoglobin, unit- gram/dL; MCV: mean corpuscular volume, unit- fL; MCH: mean corpuscular hemoglobin, unit- pg/cell; MCHC: mean corpuscular hemoglobin concentration, unit- g/dL; RDW: RBC distribution width, unit- %; F: hemoglobin F (fetal hemoglobin), unit in HPLC- %; P3: Hb HPLC P3 window, unit- %; A0: Hb HPLC A0 window, unit in HPLC- %; A2: Hb HPLC A2 window, unit in HPLC- %; C: Hb HPLC C window, unit in HPLC- %; S: Hb HPLC S window, unit in HPLC- %. The values are present as Mean ±2SD except the Reference Range, which are present as low and high normal limits.

## RESULTS

The 78 HbCC patients are comprised of 37 males and 41 females with an age ranging from 24 days to 95 years and an average age 37.9 years. Among the 243 HbSC patients, 113 of them are males and 130 are females, their ages range from 3 years to 86 years with an average age of 30.1 years. The 67 cases of HbAC include 18 males and 49 females with an age range of 3 months to 60 years and average age of 17.5 years. The HPLC and CBC results of the three groups are summarized in **Table 1**.

In the HbCC group, the average RBC count (4.7 M/ul) is within normal range (4.2-5.4 M/ul). The average hemoglobin (11.4 g/dl), MCV(68.7 fl), and MCH(24.3 pg) are lower than references (Hb 12.0-16.0 g/dL, MCV 71.4-94.6 fl, and MCH 26.6-31.6 pg), but the average MCHC(35.4 g/dl) is within normal limits (MCHC reference 30.5-35.5 g/dL).

In the HbSC group, the average RBC count (4.1 M/ul) and Hemoglobin (10.9 g/dl) are lower than normal, but the average MCV (77.5 fl), MCH (27.0 pg), and MCHC (34.8 g/dl) are within normal limits.

In the HbAC group, except that the average hemoglobin (11.8 g/dl) and MCH (26.4 pg) are slightly lower than normal limits, all other parameters are within normal range.

To further study the MCHC value in these three patient groups, cases with elevated MCHC value were separated from those with normal MCHC. Data are listed in **Table 2**.

**Table 2.** Subclassification of MCHC change in HbCC, HbSC, and HbAC.

| | | Cases | erythropenia | anemia | microcytosis | HbF increased |
|---|---|---|---|---|---|---|
| HbCC | MCHC increased | 38 | 12/38 (32%) | 21/38(55%) | 28/38 (74%) | 15/38(39%) |
| | MCHC normal | 40 | 9/40 (23%) | 25/40(63%) | 27/40 (68%) | 17/40(43%) |
| HbSC | MCHC increased | 51 | 26/51 (51%) | 42/51(82%) | 17/51 (33%) | 24/51(47%) |
| | MCHC normal | 192 | 77/192(40%) | 111/192(58%) | 25/192(13%) | 45/192(23%) |
| HbAC | MCHC increased | 1 | 0/1 (0%) | 1/1 (100%) | 0/1 (0%) | 0/1 (0%) |
| | MCHC normal | 66 | 18/66 (27%) | 37/66 (56%) | 7/66 (11%) | 6/66 (9%) |

There is no MCHC-decreased patient in all three groups. As in the table 2, in HbCC group, about half of the cases (38/78) have elevated MCHC. Furthermore, comparing the MCHC-elevated subgroup and MCHC-normal subgroup, the percentages of erythropenia, anemia, microcytosis, and elevated HbF (which could interfere the MCHC value by replacing HbCC with HbF) are similar.

In HbSC group, MCHC elevation only happens in about 20% of cases (51/243), but more patients have erythropenia and anemia that that in HbCC group. This phenomenon is possibly caused by the mutual detrimental effects between HbC and HbS and the subsequent hemolysis.[8] There are more HbF-elevated patients in MCHC-elevated subgroup (47%) than that in MCHC-normal subgroup (23%).

In HbAC group, only 1 patient has elevated MCHC (1/67), yet more than half of the patients (38/67) are anemic, although the average hemoglobin (11.8 g/dl) is only slightly lower than the reference value (12.0 g/dl).

To rule out the possibility of any bias caused by the hemoanalyzer, One month of control data from the machine were retrieved and analyzed. The hemoanalyzer ADVIA 2120 uses three manufacturer provided controls every shift: low, medium, and high controls. The one month control data showed that every run was within control and no violation of Westgard QC rules was found.

In summary, about 50% of the HbCC patients and 20% of HbSC patients have elevated MCHC, and in HbAC patients, elevation of MCHC is rare. These data are contradictory to what are in literature, that is, elevation of MCHC is universal in HbCC and HbSC, and up to 45% in HbAC.

## DISCUSSION

In this study, we analyzed the MCHC values in three patient groups, HbCC, HbSC, and HbAC. We found that MCHC was elevated in about 50% of HbCC patients, 20% of HbSC patient, and less than 2% in HbAC patients.

MCHC is a parameter about the relationship between the RBC volume and hemoglobin mass. In HbCC, RBC has certain morphological changes due to dehydration and matrix change resulting in target cell and microspherocyte in peripheral smear. The hemoglobin C, relative to hemoglobin A, has an elevated intramolecular and intermolecular attraction due to the electrical charge change by the lysine (positively charged) replacement of glutamate (negatively charged). This results in a denser hemoglobin mass in HbCC, and in oxygenating status, forming of tetragonal crystals.[13-15]

Regarding HbSC, a hydrophobic valine replacement of negatively charged glutamate at 6th position of beta chain forms the basis of HbS and the consequent RBC sickling. The presence of intra-erythrocytic HbC increases the intracellular hemoglobin concentration by loss of water and potassium, and increasing the tendency of HbS to polymerize.[4,8,16]

It has been reported that RBC density is directly related to the MCHC, and average MCHC in HbCC, HbSC, and HbAC are 38, 37, and 34 g/dl (reference range 30.5-35.5 g/dl).[8] Our data, which show that the average MCHC values in HbCC and HbSC are within normal limits, somehow do not agree with other researchers' observations. Multiple potential interference factors have been investigated and presented as follow.

1. HbF. 32/78 (41%) cases of HbCC and 69/243 (28%) cases of HbSC have elevated HbF. Persistence of fetal hemoglobin is usually caused by mutation of beta gene cluster. The percentage of expression might be as low as 10-15% or as high as 100% of the total hemoglobin.[17] HbF may alleviate the severity of certain hemoglobinopathy and therefore is selected in populations with a high prevalence of hemoglobinopathy.[17] Obviously, HbF, which has a normal MCHC, will interfere the MCHC value of accompanied hemoglobinopathy. The HbF frequencies of the three patient groups are listed in **Table 3**.

As we can see, in HbCC group, elevation of HbF almost equally distributed in MCHC-elevated (39%) and MCHC-normal patients (43%). To further analyze the potential effect of the level of HbF in MCHC, 10% of HbF is set as the boundary to separate high HbF and low HbF. Again in both MCHC-elevated and MCHC-normal subgroups, the frequencies of high or low HbF are almost equally distributed, which largely rule out the potential effect of HbF level on MCHC value.

**Table 3.** HbF in HbCC, HbSC, and HbAC.

| | MCHC | Elevated HbF frequency | HbF> 10% | HbF<10% |
|---|---|---|---|---|
| HbCC | Elevated | 15/38 (39%) | 7/15 (47%) | 8/15 (53%) |
| | Normal | 17/40 (43%) | 10/17 (59%) | 7/17 (41%) |
| HbSC | Elevated | 24/51 (47%) | 3/24 (13%) | 21/24 (88%) |
| | Normal | 45/192 (23%) | 3/45 (7%) | 42/45 (93%) |
| HbAC | Elevated | 0/1 (0%) | 0 (0%) | 0 (0%) |
| | Normal | 6/66 (91%) | 2/6 (33%) | 4/6 (67%) |

In HbSC group, elevation of HbF happens more in MCHC-elevated subgroup than in MCHC-normal subgroup (about 47% vs. 23%). But the majority of patients in both subgroups have a low level elevation of HbF (88% in MCHC-elevated and 93% in MCHC-normal subgroup). This again negates the possibility of interfering effect of HbF in MCHC value.

In HbAC group, less than 10% of patients have HbF elevation and most of the elevation cases are in the low-elevation subgroup. MCHC values of all these HbF-elevated cases are normal.

In summary, HbF elevation is relatively a common phenomenon in HbCC and HbSC patients, but its effect on MCHC value is not obvious.

2. Thalassemia. Alpha or beta thalassemia will reduce RBC hemoglobin mass and subsequently cause the decrease of MCV, MCH, and MCHC.[18] If thalassemia is compounded with HbCC or HbSC, the MCHC values of each can be reduced.[12] In HPLC, changes of fraction A2 is a sensitive marker for thalassemia, with an elevation of A2 in beta thalassemia and a decrease in alpha thalassemia.[19] But in HbCC or HbSC patients, the glycosylated hemoglobin C1C or S1C are eluted at similar retention time to that of A2, and therefore diminishes the usefulness of A2 in thalassemia diagnosis.[20] In lieu of this situation, RBC count and MCV value are instead analyzed. In thalassemia, RBC count is increased and MCV is decreased.

In HbCC group, the average RBC count (4.7 M/ul) is within normal range (4.2-5.4 M/ul). MCV (68.7 fl) is lower than references (71.4-94.6 fl). Since HbCC is also microcytic in nature, the decrease MCV cannot rule in/rule out the potential existence of thalassemia, although the normal RBC count favors minimal thalassemia effect.

In the HbSC group, the average RBC count (4.1 M/ul) is lower than normal range, and the average MCV (77.5 g/dl) is within normal limits. Considering HbSC is easy to sickle and be destroyed, the RBC count is minimally useful to determine the existence of thalassemia. The normal MCV value, however, favors against thalassemia.

In the HbAC group, the average RBC count (4.4 M/ul) and average MCV (77.5 fl) both are within normal limits, therefore thalassemia is not considered.

In summary, using RBC count and MCV, the potential effect of thalassemia on MCHC in these patient groups are not favored.

3. Hemoanalyzer. As we know, HbCC and HbSC can be rehydrated, expanding their cell volume, and subsequently decreasing MCHC. As a matter of factor, this is one of the therapeutical goals for these patients to decrease crystallization and sickling. Early hemoanlyzers, like Beckman Coulter S or Beckman Coulter S-plus, could not detect MCHC change in HbCC and HbSC.[21,22] The finding of elevated MCHC was on Technicon H1.[7] Our CBC is run on a Simens Advia 2120 hematology analyzer, which directly counts RBC numbers, measures MCV and hemoglobin concentration (MCHC). The Advia 2120 adopts a technology called iso-volumetric sphering to eliminate the shape of the cells as a variable, thereby reducing the potential for reporting erroneous results. As the name suggests, this process will not change cell volume (MCV). By the way, red cells are analyzed on the ADVIA 2120 using red laser light to measure both volume and hemoglobin concentrations on a cell by cell basis. The amount of light scattered at the low angle (2º-3º) is dependent on the volume of the cell. The amount of light scattered at the high angle (5º-15º) is related to the refractive index of the cell. This measurement provides the intracellular hemoglobin concentration of the red cell (MCHC). Interestingly, a comprehensive study which compared the performance of Beckman Coulter HL750, Bayer Advia 120, and SYSmex XE 2100 showed that the latter two had increased MCV (up to 5 fL) and hematocrit.[23] The performance of Advia 2120 with regard to other brands of hematology analyzers is not available. Therefore, any bias on MCHC created by the Advia 2120 is still waiting to be investigated.

Overall, by analyzing HbCC, HbSC, and HbAC patient groups, we found that MCHC is elevated in about 50%, 20%, and 1.5% of HbCC, HbSC, and HbAC patients. This finding is in discrepancy with what in literature, which states that MCHC is universally elevated in HbCC and HbSC patients, and nearly half of the HbAC patients. By analyzing the HbF level and CBC, we are certain that this discrepancy is not attributable to HbF and thalassemia interferences. Further research is needed to address if Advia 2120 has any systemic bias on MCHC measurement.

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**CONFLICT OF INTEREST**

NONE.

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