Staphylococcus Spp Bacteremia Complications in Acute Leukemia Patient After Chemotherapy

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Abstract

This study was designed to assess types and frequency of bacteremia with a positive blood culture for *Staphylococcus Spp* infectious etiology of 84 enrolled patients afflicted with Acute types of leukemia, after induction phase chemotherapy .Leukemia was identified by immunophenotyping with flow cytometry. The results showed that the number and percentages of B-cell Acute Lymphoblastic Leukemia (ALL) patients were higher 38(%70.4), than T-cell ALL 16(%29.6). The highest number of the ALL patients was subtype L1 35(%64.8) .While the highest number of Acute Myeloid Leukemia (AML) subtype was M4 10(% 33,3) patients. while 31(%81.6) of B-ALL has been classified into maturational stage common-B ALL because of the positivity to CD10 and the remain 7(%18.4) were at pro-B ALL maturational stage. Also CD 34 positivity was 6 (%75.5) with mean percentage of positivity (71.70) .

Isolation of bacteria by BACTEC blood culture and Identification and antibacterial susceptibility testing by VITEK2 system were done for all isolates that caused bacteremia in patients receiving induction phase chemotherapy. The results shows that after starting induction phase chemotherapy 7 isolates were observed in these patients 3(%42.9) in ALL and 4(%57.1) in AML, 5(%71.4) of these isolates were *S.aureus* and 1(%14.3) for each *S. vitulinus* and *S. epidermidis* . Benzylpenicillin was the most effective antibiotic against the bacterial isolates.

Key words: *Staphylococcus Spp* , ,chemotherapy, Leukemia, Acute Leukemia, Flowgytometry

Introduction

Acute leukemia encompasses a group of neoplasm that morphologically and immunophenotypically resemble B-lineage ,T-lineage precursor cells or myeloid stem cell, it characterized by the clonal accumulation of immature blood cells in the bone

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marrow. These abnormal cells are arrested in the lymphoblast stage of the normal maturation pathway .Aberration in proliferation and differentiation of these cells are common and normal hematopoisis is suppressed (Douglas, 2007, Swerdlow , 2008) . The most commonly used classification schemata for AML, is the French-American British (FAB) system divided AML into 8 subtypes ,M0 through to M7,based on the type of cell from which the leukemia developed and its degree of maturity. These subtypes have varying prognoses and responses to therapy(Pejovic *et al*; 2002 and Drew,2007, Barbara *et al* ,2006).

B-ALL has been classified into three different maturational stages based on the degree of differentiation of B-lineage lymphoblasts pro-B ALL CD19, cyCD79a, cyCD22, HLA-DR, CD34±, CD10-, common-B ALL CD19, cyCD79a, cyCD22, HLA-DR, CD34±, CD10+ and pre-B ALL CD19, cyCD79a, cyCD22, HLA-DR, CD34±, CD10, cyIgμ (Borowitz and Chan, 2008). And T-cell ALL can be stratified into different stages of intrathymic differentiation according to the antigens expressed Pro-T cyCD3+, CD7+, CD2- , CD1a-, CD34+/-, CD4-/CD8-, Pre-T cyCD3+, CD7+, CD2+, CD1a-, CD34+/-, CD4-/CD8-, cortical T cyCD3+, CD7+, CD2+, CD1a+, CD34-, CD4+/CD8+and medullary cyCD3+, CD7+, CD2+, CD1a-, CD34- and CD3+, CD4+or CD8+Pro-T and pre-T stages are double-negative for CD4 and CD8, while the cortical T-cell stage shows a double-positive CD4/CD8 phenotype. The medullary T-cell stage expresses only either CD4 or CD8. It may be very difficult to distinguish the rare true NK precursor ALL from T-ALL that expresses only immature markers. CD56 expression, while characteristic of NK cells, doses not exclude T-cell leukemia. Markers characteristic of T-cells, such as CD7 and CD2, may be seen in NK cell precursors (Spits et al., 1995).

Bloodstream infections represent a major cause of morbidity and mortality in cancer patients (Collin *et al* ,2001). Cancer is associated with a strongly increased risk of acquiring bloodstream infections (Laupland *et al* ,2004 and Vincent *et al* ,2009). . Cancer patients are more vulnerable to developing invasive infection due to various reasons, including an often progressive catabolic state, ulcerating lesions in mucosal surfaces and immune suppression secondary to chemotherapy, radiation, immune-modulating therapeutics and/or the malignancy itself (Safdar and Armstrong,2001). Patients with neutropenia are particularly pronemto developing bloodstream infections, with the highest risk for patients who have undergone bone marrow transplantation (Collin *et al* ,2001, Wisplinghoff *et al* ;2003) .Until the 1980s, Gram-negative bacteria were the most common cause of bloodstream infections in the western world. Since then, Gram-positive organisms have become increasingly frequent as causative agents of bloodstream infections (Martin *et al* , 2003).

The aim of this study to diagnosis ALL and AML by Flow cytometry, isolation and identification of *Staphylococcus Spp* bacteria from blood of these patients receiving induction phase chemotherapy and antibiotic sensitivity for the isolates .

Material and Methods

Blood Sample : A total of 84 blood samples were collected aseptically from enrolled groups. 16 ml adult blood and 10 ml child blood for blood culture. Each blood sample was culture by automated blood culture BACTEC 9050 . Blood volume for each vial is 8 ml for adult and 5 ml for child inoculated into aerobic vial. The same blood volume inoculated into anaerobic culture vial. Each vial was labeled with the appropriate patient information. Each positive vial was gram stained then cultured directly on blood agar for gram positive bacteria . Identification and antibiotic sensitivity for pure colony was done by VITEK 2 directly.

Bone marrow aspiration :Bone marrow samples were collected aseptically from enrolled patient groups in collaboration with senior hematopathologists at Nanakaly Hospital. 2ml bone marrow samples from patients with newly diagnosed leukemia (ALL and AML) were Immunophenotyping with flow cytometer FC500 at Nanakaly hospital for Blood Diseases with a battery of monoclonal antibodies (Abdullah, 2010).

Results and Discussion

The American Cancer Society estimated that in the U.S. 43,000 people were diagnosed with leukemia each year. About 6,000 cases were for ALL, 13,500 cases AML, most in adults.Estimation death in 2013 for ALL was 1.430, AML 10,370, (American cancer society, 2013).

Figure (1) demonstrated that the number and percentages of B-cell ALL patients were 38(70.4%), while T-cell ALL were 16(29.6%). In general, patients with B-lineage ALL have a more favorable clinical outcome than those suffering from T-lineage ALL (Jules *et al*.,2009). About 85 % of cases were precursor B-cell (pre B-cell) subtype (Chow *et al*., 2009). According to the lineage, a study by Amanda et al. (2013) found that B-cell lineage predominated (73%), but the T-cell line was statistically significant for death.

Figure (2) represented FAB classification of 54 ALL and 30 AML patients, the highest number of the cases was subtype L1 35 (64.8%) followed by L2 19 (35.2%) there is no case with L3. ALL-L1 is a more common morphological subtype might in our community because most of patients in our study were children 38/54 (70.4%) and according to FAB classification, ALL-L1 morphological subtype was the most common type in childhood accounts for over 80% of cases, whereas ALL-L2 morphological subtype was the most common type in adults, it constitute 11-14% of ALL cases in

children. The mentioned results agree with some studies found that the percentage of ALL- L1 was higher 76% than other subtypes (Kamps *et al*., 1999). Nanacaly, 2009 demonstated that commonest morphological subtype in Erbil in 2008-2009 was L1. And Ishfaq *et al*. (2012) were observed 68 %, 32% for L1 and L2, respectively, and disagree with (Al- Barazanch *et al*., 2005) who found that ALL-L2 morphological subtype was more common type in both children and adults 87.2% and 92% respectively than L1 morphological subtype.

The highest number were M4 10 (33.3%) patients, followed by M1 7(23.3%), M3 6(20%), M2 were accounted for 3(10%) of all AML patients . M5 and M6 were 2 (6.7%) patients which are the lowest number of AML subtypes. While there was no case of M0 and M7 in this study.

Of the overall 1686 evaluable cases 400 (23.7%) were diagnosed AML-M4 (Pulsoni et al., 2008). Other researchers demonstrated that the most common subtype was M1 (35%) followed by M3, M4, M2, M6 31%,15%,15% and 4%, respectively. None of the patients were diagnosed as M5, M7 or M0 subtype (Asif et al., 2011).

In our study the lymphoblasts of all B-cell ALL patients 38(100) were positive for the B-cell markers CD19 and cyC79a with a mean percentage of positivity76.57 and 64.05 respectively as shown in Table (1) and only 26(68.4) of patients were expressed CD 34 (36.33), While most of patients expressed CD 10 (73.33) therefore, 31(81.6) of B-ALL has been classified into maturational stage common-B ALL because of the positivity to CD10 and the remain 7(18.4) were at pro-B ALL maturational stage.

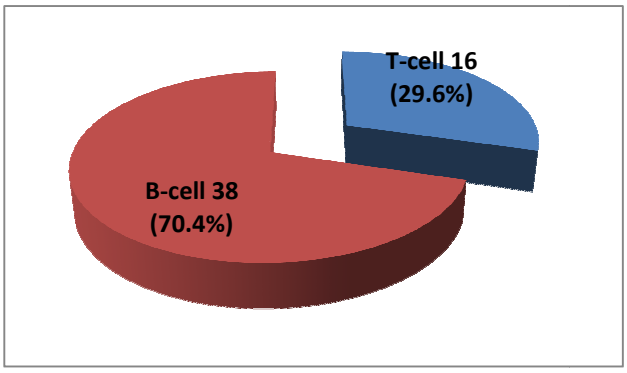
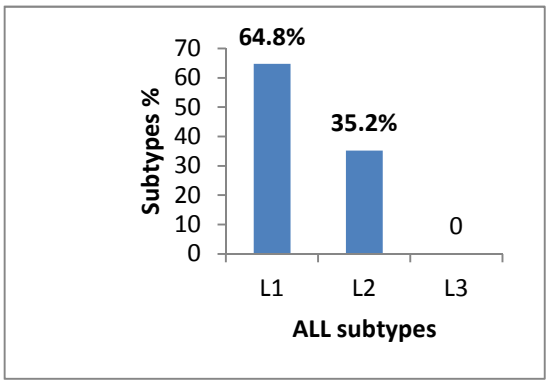
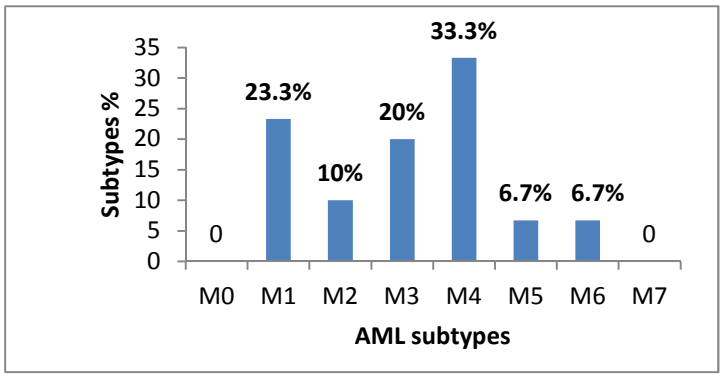


Figure (1): Types of the cells



(a)



(b)

Figure (2): Subtypes of a)ALL and b)AML

Table (1) Immunophenotype and CD marker of B- Cell ALL leukemia

CD marker of B-cell ALL	N(%)of positive case	Mean % of positivity	Rang of positivity	conjugated fluorochromes
cyCD 79a	38(100)	76.57	42-98	PE
CD 19	38(100)	64.05	20-95	ECD
CD 34	26(68.4)	36.33	22-55	ECD
CD 10	31(81.6)	73.33	22-99	PE

The Table (2) represents the CD marker of T- cell ALL leukemia. The number and percentage of patients whose lymphoblasts expressed CD1a are 10(62.5), CD2 11(68.75) with mean percentage of positivity 67.50 and 62.33 respectively, while all patients expressed CD7 and CD3 on their blast cell 16(100) with mean percentage of positivity 91.11 and 81.65 respectively, while CD 34 positivity was 6(75.5) with mean percentage of positivity (71.70) which means that the lymphoblast of most of case is at

cortical T stage, the results also indicate that the lymphoblasts of some cases were negative for CD1a and CD2 and stages of intrathymic differentiation according to the antigens expressed are Pro-T-ALL and /or pre-T- ALL, medullary T-stage ALL was also present in some case which did not expressed CD1a and CD34.

Table (2) Immunophenotype and CD marker of T- Cell ALL leukemia

CD marker of T-cell ALL	N(%)of positive case	Mean % of positivity	Rang of positivity	conjugated fluorochromes
CD1a	10(62.5)	67.50	53-82	PC5
CD 2	11(68.75)	62.33	40-95	ECD
CD 5	14(87.5)	83.33	80-90	FITC
CD 7	16(100)	91.11	80-99	PC5
CD 3	16(100)	81.65	73-90	PE
CD 34	6(75. 5)	71.70	70-83	FITC/PF

Table (3) describes the CD marker of AML leukemia. The number and percentage of patients which the myeloid cells expressed cyMPO 27(90) with mean percentage of positivity 59.38,while all patients expressed CD33 and CD13 on their blast cell 30(100) with mean percentage of positivity 59.81 and 30.83 respectively, while CD 117,CD15, CD34, CD64, CD36 and CD14 positivity were 21(70), 13(43.33), 8(26.67), 10(33.33), 11(36.67) and 11(36.67) respectively, with mean percentage of positivity 48.33, 24.66, 45.11, 56.22, 43.14 and 23

Most of patients were myelomonoblastic M4 subtype because blast populations expressed CD13, CD33, and CD15 antigens with monocytic differentiation markers CD14 and CD36, CD64. Blast cells of some case expressed CD marker for M1 like cyMPO, CD117and CD34, for M3cMPO and CD13, M2 CD marker were CD34,CD117and CD15, while in M5 were CD14 and CD36 and finally in M6 were CD13, CD33, and CD117 with cy MPO.

Table (4) shows distribution of *Staphylococcus Spp* isolates in blood after induction phase chemotherapy according to types of leukemia, there was no any isolates of *Staphylococcus Spp* in the blood of these patients before starting induction phase chemotherapy. While after starting induction phase chemotherapy 7 isolates were observed in acute leukemia patients 3(42.9) in ALL patients and 4(57.1) in AML, 5(71.4) of these isolates were *S.aureus* and 1(14.3) for each *S. vitulinus* and *S. epidermidis*.

Table (3) Immunophenotype and CD marker of AML leukemia

CD marker of AML Leukemia	N(%) of positive case	Mean % of positivity	Rang of positivity	conjugated fluorochromes
cyMPO	27(90)	59.38	24-92	
CD 33	30(100)	59.81	21-94	FITC
CD 13	30(100)	30.83	20-45	PC5
CD 117	21(70)	48.33	20-70	PE
CD 15	13(43.33)	24.66	20-30	PC5
CD 34	8(26.67)	45.11	20- 69	ECD
CD 64	10(33.33)	56.22	25-90	PE
CD 36	11(36.67)	43.14	20-88	FITC
CD14	11(36.67)	23	21-25	ECD

Table (4) Number of *Staphylococcus Spp.* in the blood before and after induction phase chemotherapy.

Leukemia types	*S. aureus*		*S.haemolyticus*		*S.lentus*		*S.vitulinus*		*S.epidermidis*		*S.hominis*		*S.warneri*		Total	
	Before															
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
ALL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AML	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leukemia types	After															
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
ALL	2	40	0	0	0	0	0	0	0	0	1	0	0	0	3	42.9
AML	3	60	0	0	0	0	1	100	0	0	0	0	0	0	4	57.1
Total	5	71.4	0	0	0	0	1	14.3	1	14.3	0	0	0	0	7	100

Gram-positive bacteria represented the majority of bloodstream isolates, *S.aureus* accounted for more than half of these isolates followed by CoNS, while *S. epidermidis* was a major component of the skin flora, that most often contaminates devices that provide adirect access to the blood stream (Wade *et al.,*1982). The differences might be explained, at least in part, by the shift towards gram-positive bacteria which in turn was due to factors such as the wide use of indwelling central catheters (Edmond *et al*.,1999). Infections by gram-negative and gram-positive bacteria were documented in the blood of ALL patients (Afzal *et al*., 2009). Similar results were reported by Feusner and Hastings (2002). The results also agree with Lehrnbecher *et al*. (2004) who observed that overall 252 isolates from bloodstream infections of pediatric AML patients 203 (80.6%) were gram-positive bacteria CoNS, *S.aureus, Streptococci,Enterococcus* and unspecified gram positive cocci . Infections caused by gram-positive cocci, mainly *Staphylococcus epidermidis* and Streptococci in febrile neutropenic cancer, have been pointed out by lastersky *et al*., 2007.

Table (5) illustrates the range of minimum inhibition concentration (MIC) of 14 antibiotics *Staphylococcus Spp* isolates after induction phase chemotherapy .The

lowest/highest MIC range against isolates of *S.aureus* was ≥0.5-≥2 /≥20-≥160 µg /ml for Benzylpenicillin and Teicoplanin /Trimethoprim Sulfamethoxazole

While the lowest /highest MIC range against isolates of *S.vitulins* after chemotherapy was ≥0.1-≥1/≥20-≥320µg/ml for Nitrofurantoin /Trimethoprim Sulfamethoxazole. The lowest /highest MIC range against isolates of *S.epidermids* was ≥2-≥32/≥80-≥320µg/ml for Benzylpenicillin and Erythromycin/ Trimethoprim Sulfamethoxazole. The results referred that there were appreciable differences in the MIC against organisms isolated from cancer hospitalized patients. Benzylpenicillin was the best antibiotic for the treatment of *Staphylococcus Spp* bacterial infection because the lowest MIC of this agent affected on most types of these isolates after induction phase chemotherapy followed by Teicoplanin then Nitrofurantoin. While Erythromycin inhibited *S.epidermids* .

Table (5) Minimum inhibitory concentration MIC of antibiotic against *Staphylococcus Spp* bacteria after induction phase chemotherapy

MIC of antibiotic µg/ml	isolates		
	S.aureus	*S.vitulins*	*S.epidermids*
Benzylpenicillin	≥0.5-≥2	≥16 - ≥32	≥2-≥ 32
Oxacillin	≥1 - ≥8	≥ 2 -≥ 32	≥32-≥128
Gentamicin	≥2 - ≥ 8	≥ 4- ≥ 32	≥32-≥256
Tobramycin	≥2- ≥ 32	≥16-≥ 64	≥16 -≥128
Levofloxacin	≥4 -≥	≥32-≥ 64	≥8 - ≥256
Moxifloxacin	≥0.5- ≥4	≥4 -≥32	≥8- ≥ 64
Erythromycin	≥0.2-≥32	≥16-≥ 32	≥2-≥ 32
Teicoplanin	≥0.5-≥2	≥ 0.2 - ≥2	≥1 -≥ 64
Vancomycin	≥ 8-≥ 128	≥2 - ≥4	≥4 -≥ 128
Teracycline	≥8 -≥64	≥1-≥ 64	≥8 -≥256
Nitrofurantoin	≥ 16-≥ 64	≥0.1-≥1	≥32 -≥ 64
Rifampicin	≥ 0.5-≥32	≥0.5-≥16	≥ 32-≥128
Trimethoprim/ Sulfamethoxazole	≥20-≥ 160	≥20-≥ 320	≥80-≥ 320

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