A RETROSPECTIVE CHART REVIEW OF CEREBROSPINAL FLUID CHARACTERISTICS OF INFANTS WHO PRESENT TO THE EMERGENCY DEPARTMENT WITH FEVER: ESTABLISHING NORMAL VALUES

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A RETROSPECTIVE CHART REVIEW OF CEREBROSPINAL FLUID CHARACTERISTICS OF INFANTS WHO PRESENT TO THE EMERGENCY DEPARTMENT WITH FEVER: ESTABLISHING NORMAL VALUES

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Bacterial and viral meningitis are public health concerns as they are contagious and highly fatal without treatment. Newborn infants are at high risk for bacterial and viral meningitis. The onset of the infection is rapid and without quick diagnosis and treatment, many infants will die. Diagnosis requires the positive identification of the causative agent through culture or the use of polymerase chain reaction (PCR) from specimens of cerebrospinal fluid (CSF). Because these tests can take hours or days to perform, it is important identify children who have a higher likelihood of serious bacterial or viral infection so that empiric therapy can be initiated while awaiting further results. Previous studies have indicated that CSF characteristics can be accurate early predictors of viral and bacterial meningitis. Although CSF characteristics have been established for infection, normal values for infants less than 60 days of age are still not clear. To improve characterization of CSF values for infants, this study set to answer three questions: Is there a temporal relationship for CSF WBC, glucose, and protein? What are the means and confidence intervals for the means for each of these variables? What is the range of normal values that a physician could expect to find in infants less than two months of age? This study involved three independent retrospective chart reviews over a 15-year period to identify infants less than two months of age who presented to The Children's Hospital of Pittsburgh emergency department with fever and had lumbar punctures performed but were not found to have bacterial or viral meningitis. For CSF WBC and protein, the data from the three cohorts

were pooled and a single set of reference values was generated for infants less than two months of age. CSF glucose values were not pooled due to differences that existed between the cohorts and reference values were calculated for the cohorts individually. CSF white blood cell (WBC), glucose, and protein values were analyzed to answer our three study objectives. A temporal trend was found for CSF WBC and protein with values being highest during the first weeks of life. CSF glucose values did not change with time. These values will be potentially valuable reference tools in emergency departments for physicians who are faced with decisions regarding care and treatment of febrile infants.

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PREFACE

To LaToya – my fellow student, partner in crime, and best friend.

Our Time in Grad School:
Secrets, Laughs, Hard Work, No Sleep
Friendship Saw Us Through

1.0 INTRODUCTION

1.1 DESCRIPTION OF THE PROBLEM

Lumbar puncture has long been the standard of care for infants less than 60 days of age who present with fever in emergency departments[1]. Although the procedure has its risks, it is the best way to determine whether the infant has meningitis. Administering antibiotics without performing a lumbar puncture can lead to complications, delaying diagnosis of bacterial meningitis and can make analyzing cerebrospinal fluid (CSF) diagnostic values difficult[2]. Bacterial and viral meningitis, which are inflammation of the meninges caused by bacteria or virus, respectively, have very high mortality rates in babies - as high as 20%[3]. Early identification of meningitis can result in better outcomes by leading to earlier treatment of the infection and better management of the infant. Currently, significant amounts of time are required to complete bacterial and viral cultures and other diagnostic tests on the CSF. Therefore, a rapid predictor of meningitis is needed to help accelerate the process of identifying infants who are in need of treatment. In 1989, Spanos *et al*. made the observation that the initial values of the CSF, including glucose, protein, and white blood cell (WBC) levels, can be good predictors of disease outcome[4]. Although no single factor was able to predict whether or not an infant will test positive for infectious meningitis, the three taken together were able to predict 99% of cases of infection.[4,5] Although in general there are certain values for CSF WBC,

glucose, and protein which are more likely to be associated with bacterial and viral meningitis, it is uncertain just how high or how low these values need to be to warrant action or empiric therapy. In other words, values for normal infants without meningitis need to be determined in order to better direct treatment. Despite previous studies attempting to identify the CSF characteristics of healthy infants, no study yet has incorporated stringent enough inclusion criteria, included a substantial number of infants for analysis, or has stratified infants in appropriate age categories to provide values that have clinical relevance. This study fills this void in the literature by including infants less than eight weeks of age who were not born prematurely, had no previous history of seizure, did not have a traumatic lumbar puncture (LP; >1000 red blood cells [RBCs] /mm^3), and had negative blood, urine, and CSF cultures for microorganisms. Our hypothesis for this study is that significant variation exists within CSF WBC, glucose, and protein for infants depending on age by week. Specifically, we want to answer three objectives: Does a significant temporal trend exist for these variables, what are the means and confidence intervals for CSF WBC, glucose, and protein, and what is the expected range of values for these variables. These reference values will serve as a potentially valuable predictive tool for physicians treating febrile infants with suspected meningitis before CSF culture and diagnostic tests can be completed.

1.2 CEREBROSPINAL FLUID

CSF is a clear, colorless fluid that is primarily made in the choroid plexus in the lateral ventricles by modified ependymal cells[6]. These cells do not passively or actively transport CSF following its production, rather they secrete it after it is made. Following the filtration of blood that is the

precursor of CSF, the CSF is secreted into the lateral ventricles of the brain[6,7]. Following its production in the choroid plexus, the CSF flows through the interventricular foramen of Munro to the third ventricle. From there, it flows to the fourth ventricle and through the foramen of Luschka and Magendie to reach the subarachnoid spaces.[7] The anatomical locations and pathways of CSF flow can be seen in Figure 1.

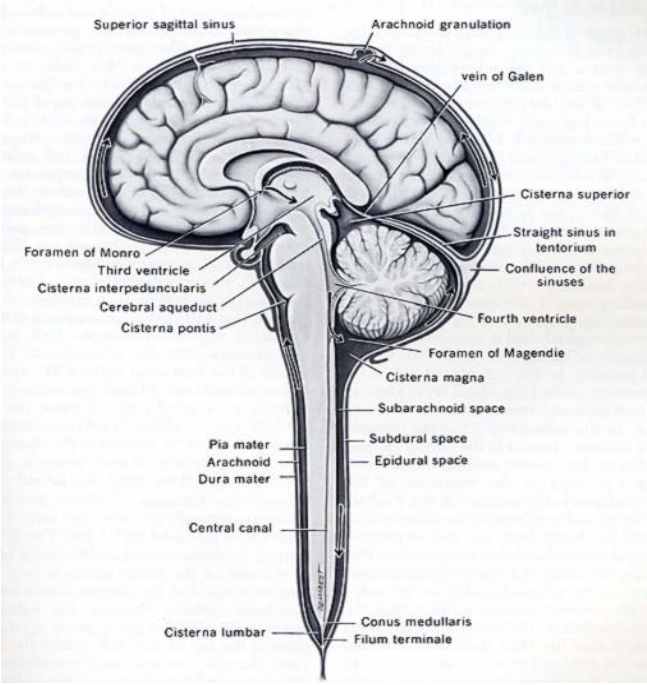

Figure 1: Pathways of the Formation of the Cerebrospinal Fluid[6].

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The subarachnoid space, the region between the pia mater and the arachnoid mater, surrounds the brain, ventricles, and spinal cord[6,7]. The subarachnoid spaces and the ventricles contain approximately 140 mL of CSF, and it is estimated that it is remade every five to seven hours. This constant renewal allows for a lumbar puncture to provide an accurate "snapshot" of what is present in the CSF in terms of cell counts, glucose, and protein levels.

The CSF serves many physiologically significant roles. Primarily, its role is to provide a cushion for the brain – it provides buoyancy and reduces the weight of the brain by nearly 95%[6,7]. The CSF is a sterile fluid and has limited access to cells, proteins, and metabolites. This limited access is maintained by the "blood-brain barrier", which is the system that screens the particles that are allowed access to the brain. The blood-brain barrier is important not only for necessary metabolites but also for access of cells in normal and pathological situations. The choroid plexus is largely made up of blood vessels and specialized endothelial cells. The barrier between these cells and the subarachnoid space is called the brain-CSF barrier. It is a secondary barrier to the blood-brain barrier, and also functions in preventing access of blood metabolites and cells to the CSF[8]. Superficially, the ependymal cells appear like cells in the small intestine, which are arranged in villi and designed for absorption. A picture of these specialized cells can be seen in Figure 2.

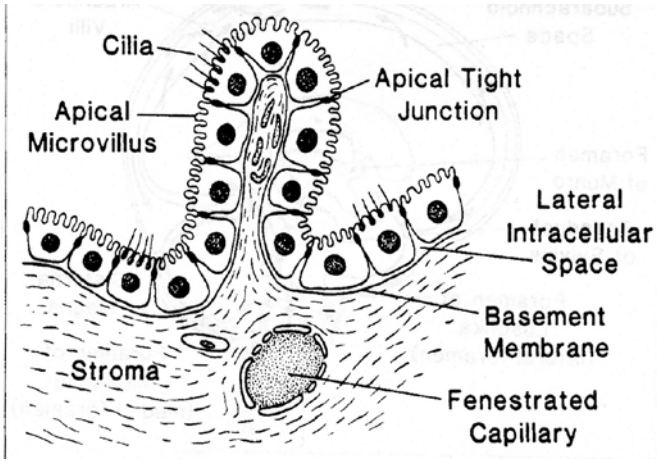

Figure 2: Epdendymal Cells are Responsible for the Formation of CSF[7].

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The blood-brain barrier is composed of the capillaries in the brain and the astrocytes, specialized support cells in the brain. The endothelial cells that make up the capillaries in the brain are different from those seen in other blood vessels, however. They form tight junctions and are bound tightly by astrocytes. A picture of the blood brain barrier can be seen in Figure 3.

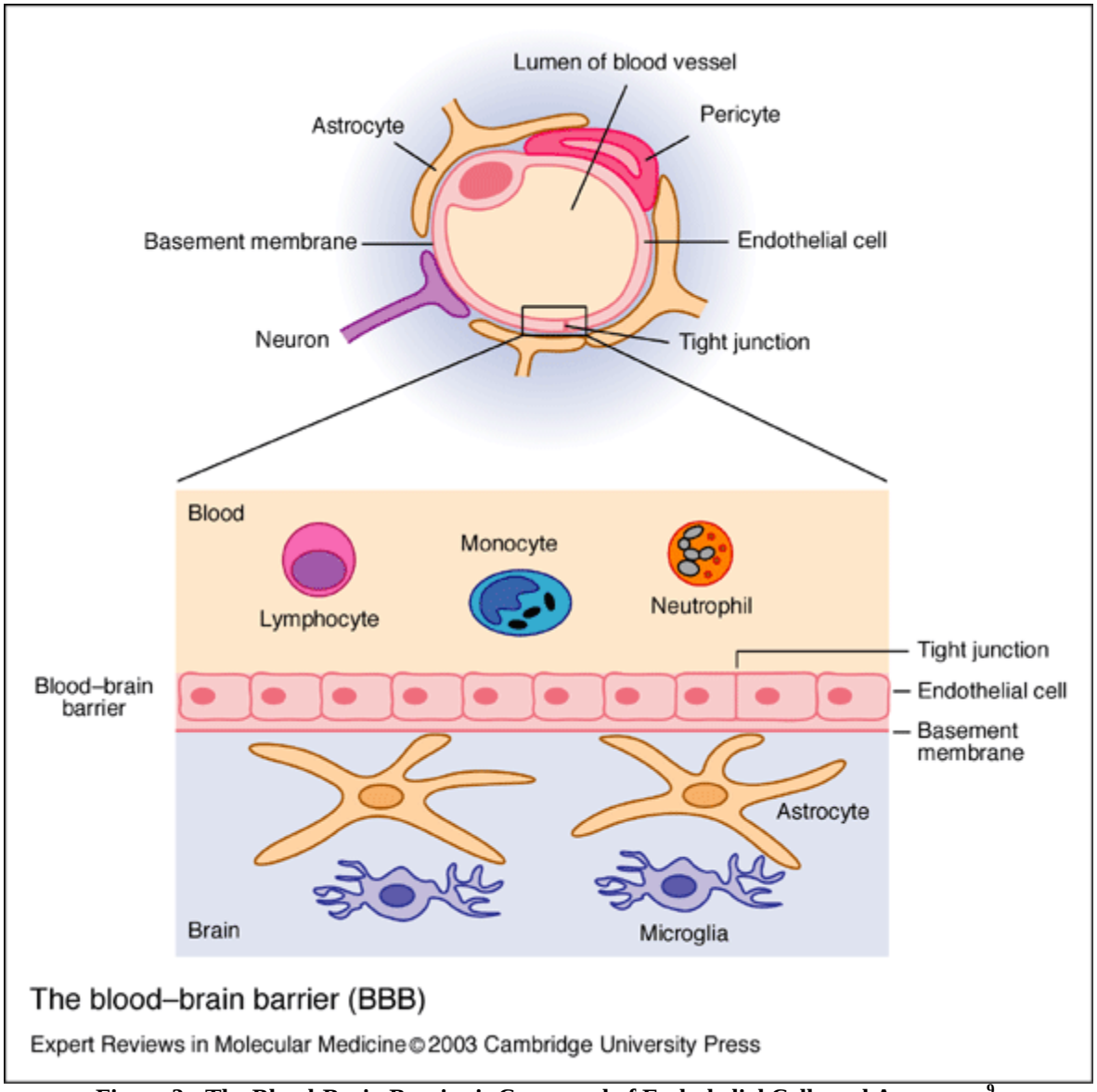

Figure 3: The Blood-Brain Barrier is Composed of Endothelial Cells and Astrocytes[9].

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Besides having tight junctions, the endothelial cells in the brain capillaries also differ from other endothelial cells based on their cellular machinery. "Intracellular clefts, pinocytotic vesicles, and fenestrae, which readily allow for transcapillary exchange in most systemic capillaries, are virtually not seen in normal brain endothelial cells"[10]. It is thought that this lack of machinery is what accounts for the exclusivity of the blood-brain barrier. A comparison of general endothelial cell transport and brain endothelial cell transport mechanisms can be seen in Figure 4.

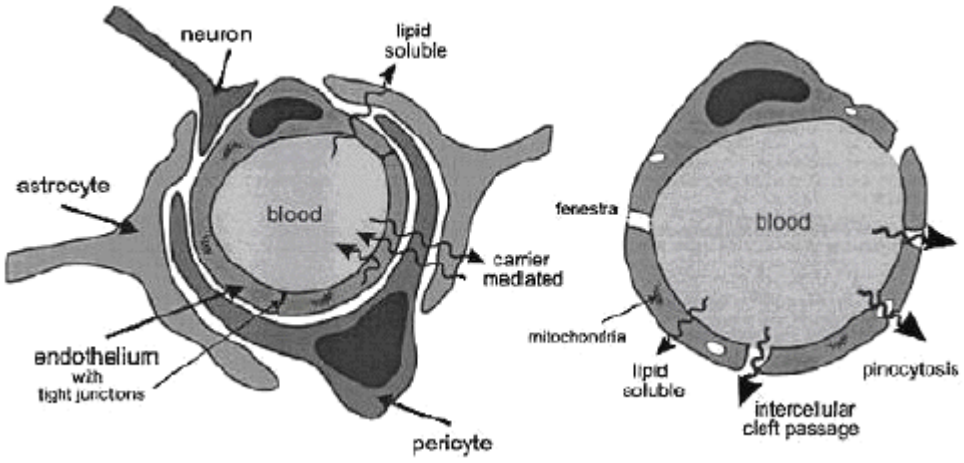

Figure 4: A Comparison of the Mechanisms of Transport between General Endothelial Cells (right) and Brain Endothelial Cells (left)[8].

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For most macromolecules, their movement across the blood-brain barrier depends on their molecular weight. The smaller the molecular, the more likely it will be able gain access to the brain. Regulation of glucose concentration in the CSF is dependent upon facilitated diffusion, while protein transfer is dependent upon ultrafiltration, diffusion (if the proteins are small enough), or pinocytosis (for larger proteins)[7]. The majority of cells and larger particles (such as

bacteria) are prevented from reaching the brain by the intricate placement of tight junctions between astrocytes and endothelial cells that compose the blood brain barrier[7,10].

There are times during development that the blood brain barrier does not have such stringent passage restraints. During infancy, the blood brain barrier has been found to be more permeable than later in life. Although it is not certain why this increased permeability exists, it may be due to the "immaturity" of the junctions between the endothelial cells and astrocytes. This can allow for more migration of cells and proteins from the blood into the CSF following birth. It is also possible that the "trauma" experienced during birth can cause some inflammation to the meninges, which additionally allows for more transport of molecules that are normally not present in the adult CSF.[10-14] It is assumed that glucose levels are higher in the first months of life because of the immaturity of the glucose exchange mechanisms[7]. Regardless of the cause, CSF characteristically has more white blood cells (WBCs), protein, and glucose during the first two months of life as compared to babies older than two months, children, and adults. These differences in CSF composition appear to decrease after about eight weeks of life, after which the CSF constituents begin to approach levels that are experienced during times of normal adult health[10].

The relative exclusivity of the blood brain barrier can also be affected by infection. Meningitis, generically defined as the inflammation of the meninges, can lead to increased permeability in the barrier. This increased permeability allows white blood cells, high molecular weight proteins, and other macromolecules to move more easily across the blood brain barrier and into the CSF. One of the classic signs of meningitis is pleocytosis – an increased WBC count in the CSF. In both bacterial and viral meningitis, pleocytosis and increased protein are present, although to varying degrees. Glucose concentrations, however, do not follow the same

pattern in bacterial and viral meningitis[15]. During bacterial meningitis, CSF glucose levels are lower than in normal homeostasis[10]. Although the reasons for this are not clear, it is suggested that as more cells and larger macromolecules enter the CSF, the ependymal, endothelial and migratory cells need more energy and thus perform greater amounts of glycolysis. Additionally, if the meningitis is caused by bacteria, the presence of bacteria in the meninges and the CSF can also account for a decrease in glucose. The combination of these factors may account for the reduction in normal levels of glucose during a bacterial infection in infants, children, and adults.[10] During viral meningitis, however, glucose levels the same or higher than normal. This could be because viruses do not require the use of supplementary glucose. Additionally, because WBC levels are not as high in viral meningitis as compared to bacterial meningitis, there are not as many cells requiring glucose. Despite these observations, the exact mechanisms and signaling pathways that lead to increased and decreased glucose transport during different types of meningitis are still largely not understood. A summary of these characteristic trends is seen in Table 1.

Table 1: Typical CSF Findings in Infectious Meningitis[15].

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Cause of meningitis	White blood cell count (cells/mm^3)	Predominant cell type	CSF:serum glucose (normal 0.5)	Protein (g/l) (normal 0.2-0.4)
Viral	50-1000	Mononuclear (may be neutrophilic early in course)	>0.5	0.4-0.8
Bacterial	100-5000	Neutrophilic (mononuclear after antibiotics)	<0.5	0.5-2.0
Tuberculous	50-300	Mononuclear	<0.3	0.5-3.0
Cryptococcal	20-500	Mononuclear	<0.5	0.5-3.0

Meningitis has many different causes, both infectious and non-infectious. The causes of infectious meningitis will be considered in the next section.

1.3 MENINGITIS DUE TO INFECTION

In general, meningitis is a generic term which refers to inflammation of the brain and spinal cord's coverings – the meninges. The brain and spinal cord are surrounded by three meninges – the closest to the brain is the pia mater, the central covering is the arachnoid mater, and the outer is the dura mater. A picture of the relation between the three meninges can be seen in Figure 5. The pia mater is a thin connective tissue membrane that closely surrounds the brain and spinal cord. The arachnoid mater surrounds the brain more loosely and also extends along the roots of

cranial and spinal nerves. The dura mater is the tough outer covering that is attached firmly to the inside of the skull.[7]

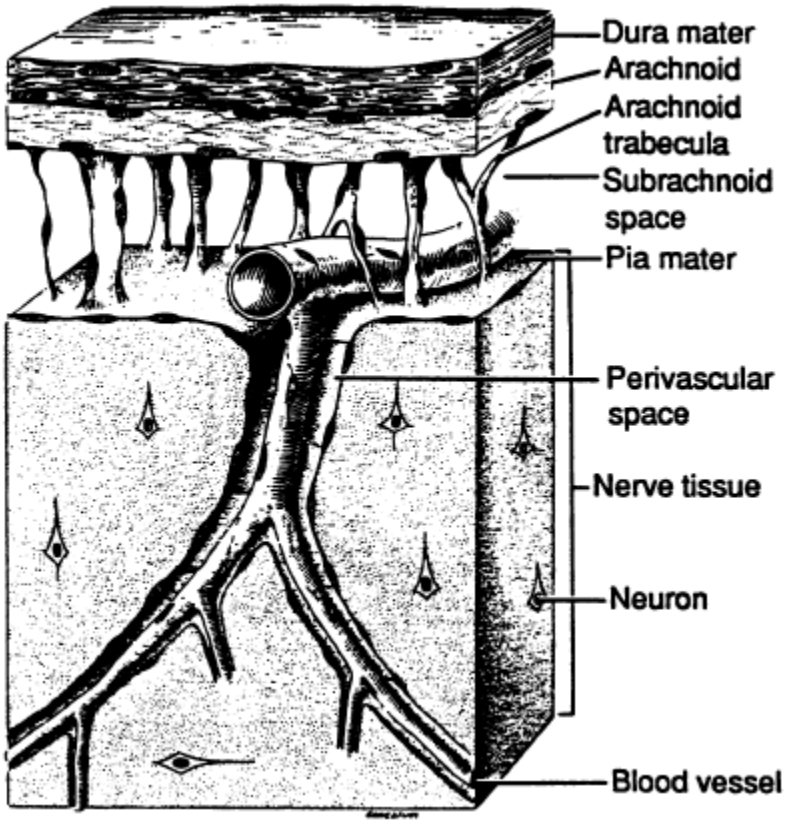

Figure 5: The meninges of the brain and spinal cord[16].

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The causes of meningitis are numerous and include trauma, complications from drug use, bacterial infections, viral infections, and fungal infections[17]. The majority of causes of meningitis are due to bacteria and viruses. For this study the following definitions will be used:

- Proven bacterial meningitis: positive CSF bacterial culture
- Viral meningitis: positive CSF viral culture or PCR test

- Presumed bacterial meningitis: CSF pleocytosis with a positive blood culture, and a negative CSF culture

The causes and rates of death of bacterial and viral meningitis vary in populations depending on the age of the patient. In neonates and infants, the rate of bacterial and viral meningitis is between two and 10 per 100,000 live births[18]. This rate is higher than at any other time in a person's life. The fatality rate of meningitis also varies due to the causative agent and the child's age. It has been postulated that without effective treatment the death rate can approach 100%[3]. In infants who receive treatment, however, the death rates are approximately 20%.[3,18] Even in children who survive, about 15% of these have long-term effects from the infections including deafness, seizures, learning disabilities, and lower intelligence.

The major causes of bacterial meningitis in young infants are Group B Streptococcus, *Haemophilus influenza type B, Listeria monocytogenes, Escherichia coli*, *Streptococcus pneumonia*, and *Neisseria meningitidis*[3,18]. In developed countries, the rates of meningitis due to Group B Streptococcus and *H. influenza* have decreased dramatically since the introduction of vaccines[19]. The majority of newborn bacterial meningitis is transmitted vertically, often through direct contact with or inhalation of bacterial strains present in the mother's vaginal and intestinal tracts. Bacterial meningitis in infants is often a result of septicemia. Although septicemia is uncommon in infants, only 1 to 8 per 1,000 live births, the rate of bacterial meningitis as a result of septicemia is 25%[3].

Viral meningitis and aseptic meningitis are terms that are sometimes used interchangeably, although aseptic meningitis is a more general term that implies that the symptoms of meningitis are present although no bacteria can be isolated from CSF or blood cultures[3]. Frequently, aseptic meningitis is caused by viruses. Tests for diagnosing meningitis

caused by viruses have advanced significantly, and polymerase chain reaction (PCR) assays are now available for the rapid diagnosis of many viral pathogens. In infants, the most common causes of viral meningitis are enteroviruses, Epstein-Barr Virus, Varicella-Zoster Virus, mumps virus, herpes simplex virus, and human immunodeficiency virus (HIV) [3,15]. The major causes of bacterial and viral meningitis are summarized in Table 2.

Table 2: The Common Causes of Infectious Meningitis in Infants.

Common Causes of Infectious Meningitis in Infants	
Bacterial	Viral
Group B Streptococcus	Enterovirus
Streptococcus pneumoniae	Epstein-Barr Virus
Haemophilus influenza type B	Varicella-Zoster Virus
Neisseria meningitidis	Mumps Virus
Listeria monocytogenes	Herpes Simplex Virus
Escherichia coli	Human Immunodeficiency Virus (HIV)

The clinical manifestations of meningitis are a sore or stiff neck, fever, headache, the inability to tolerate bright lights or noises, and in some cases, a rash on the torso[18]. In adult populations, symptoms of meningitis are easier to detect because the patient is able to tell the physician what is causing discomfort. In infants, however, most physicians have to rely on the parents' observations. Fever, irritability, and not wanting to feed are the most common symptoms of meningitis in infants[3]. These non-specific symptoms make clinical assessment difficult. The physician has to decide whether to perform a lumbar puncture, the diagnostic test for confirming meningitis. However, because of the relatively high rates of meningitis in infants, physicians are often inclined to perform a lumbar puncture and collect CSF to err on the side of caution.

1.4 LUMBAR PUNCTURE IN INFANTS

As previously stated, lumbar puncture is a common diagnostic tool in the identification of bacterial and viral meningitis. The procedure, although painful for the infant and not without risk of compliciations, is often necessary. Although there has been some debate as to whether this procedure should be common in the evaluation of febrile infants, a retrospective study done by Visser and Hall found that the removal of lumbar puncture from emergency workups would have led to 15% of cases of meningitis missed in febrile infants.[1,20]

Lumbar puncture involves using a needle that is inserted into the subarachnoid space of the spinal cord to collect cerebrospinal fluid (CSF). The overall procedure is similar to the one performed in adults but there are some key precautionary differences when performing a lumbar puncture on an infant. Primarily, the positioning of a baby differs from the positioning of an adult and is very important to ensure a safe and sufficient collection of fluid. Adults and older children undergoing the procedure are advised to position themselves in the fetal position. Infants, however, should be positioned so that their legs are perpendicular to the rest of the body and the back is slightly curved[1]. This positioning can be seen in Figure 6.

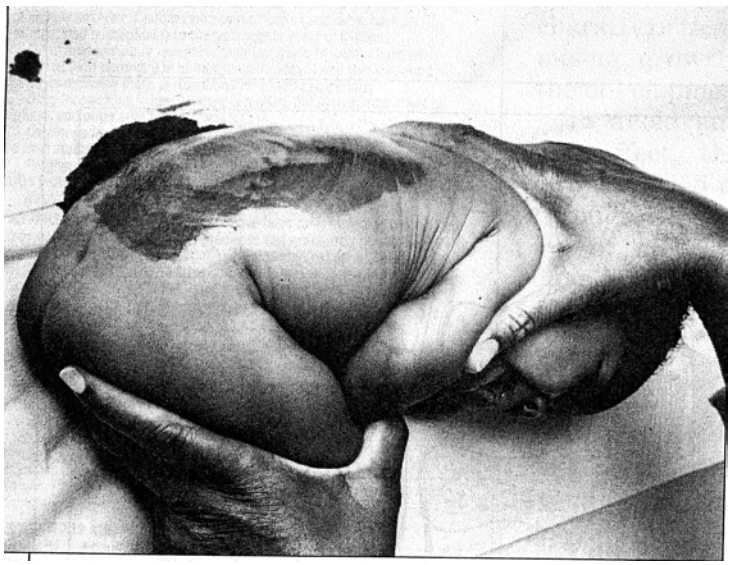

Figure 6: The Proper Positioning of an Infant for Lumbar Puncture[1].

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This position opens the spaces between the lumbar vertebrae and allows for the easiest access of the needle[1]. In most cases, the needle is inserted between the L3/L4 or the L4/L5 vertebrae. A 'pop' may be felt when the needle penetrates the dura and arachnoid mater and enters the subarachnoid space. However, this 'pop' should not be depended upon in infants, as it may be very subtle or absent[1]. The final positioning of the needle in the subarachnoid space can be seen in Figure 7.

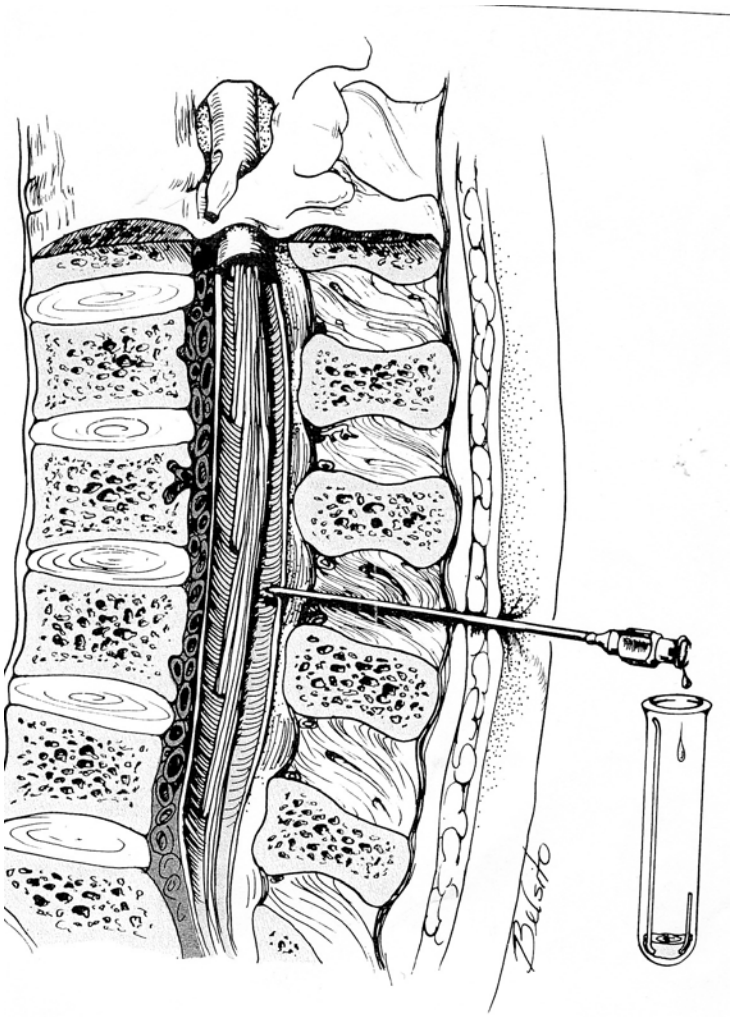

Figure 7: The Proper Positioning of the Needle when Performing a Lumbar Puncture[1]

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When performing a lumbar puncture, the clinician must be careful not to overshoot the subarachnoid space and enter the underlying tissue. This can cause a bleed into the CSF, which is more commonly referred to as a traumatic lumbar puncture. Quantitatively, a traumatic lumbar puncture is defined as being >1000 red blood cells (RBCs)/mm^3 of CSF. Because of the difficulties in performing a lumbar puncture in infants, approximately 20% of these procedures result in a traumatic lumbar puncture[1]. Besides providing discomfort to the infant, a traumatic

lumbar puncture introduces components into the CSF that can obscure the analysis. Although formulas have been developed to help "correct" for WBC counts in cases of traumatic lumbar puncture, no such formulas have been developed to help correct for glucose and protein concentrations.

In order to perform all of the necessary analyses on the CSF, a minimum of 3mL of fluid must be collected from a lumbar puncture. In general, tests must be performed within 30 minutes following collection or the cells present in the CSF will begin to lyse. A drop of CSF is needed to determine total WBC and RBC counts by using a Fuchs-Rosenthal counting chamber[21]. Samples are also used to determine the CSF protein and glucose concentrations using reagent strips similar to those used to measure protein and glucose levels in the blood or urine. If a differential count of the cells is desired, the CSF is centrifuged and then a Wright stain is applied to characterize the different distribution of white blood cells[21]. A Gram stain to check for the presence of bacteria is also commonly performed and, although this stain can be definitive, its success is dependent on the number of bacteria present in the CSF. Concentrations of $\leq 10^3$ colony forming units(cfu)/mL will only give a positive gram stain 25% of the time, concentrations between 10^3 and 10^5 cfu/mL give a positive gram stain 65% of the time, and concentrations $>10^5$ cfu/mL give a positive gram stain 97% of the time[22]. In addition, besides the concentration of bacteria present in a sample, the causative agent itself can also influence the success of identifying the presence of infection using a gram stain. As long as the colony forming unit criteria are met, 90% of cases caused by *Streptococcus pneumonia*, 86% of cases caused by *Haemophilus influenza*, 76% of cases caused by *Neisseria meningitides*, 50% of cases caused by gram-negative enteric bacilli, and 33% of cases caused by *Listeria monocytogenes* can be identified from gram stain results[22]. Because of these reasons, it is very important to perform

cultures and other diagnostic tests in addition to the initial descriptive analyses previously discussed.

Besides these rapid tests, additional tests should be done on the CSF to confirm the presence of an infection. The CSF should be cultured and tested for a variety of bacterial and viral pathogens. Standard culture technique for bacteria involves using a 5% blood agar, chocolate agar, and enrichment broth[23]. The 5% blood agar and chocolate agar plates serve to isolate true pathogens causing bacterial meningitis, whereas the enrichment broth serves as a control. *Streptococcus pneumonia* and *Listeria monocytogenes* will be able to grow on the 5% blood agar. Chocolate agar is used for the isolation of *Haemophilus influenza* and *Neisseria meningitides.* If there is growth in the broth but not on the plates, it is likely that the growth seen in the broth is not a true pathogen and is indicative of human error or contamination from the skin[23]. For culturing viruses, the frequently used standard are cultures using human diploid fibroblasts, primary monkey kidney, Buffalo monkey kidney, and rhabdomyosarcoma cell lines[14]. The use of several cell lines ensures isolation of a wide variety of viruses, including enteroviruses, herpes simplex I and II, and mumps virus[14]. In the case of viral pathogens, PCR tests have been developed for the CSF to accurately confirm the presence of infection. These tests are far more accurate than the viral cultures that were commonplace in the past[15]. Currently, PCR testing is available for enteroviruses and herpes simplex viruses at the Children's Hospital of Pittsburgh. A summary of these procedures and other PCR tests can be seen in Table 3.

Table 3: Diagnostic Tests for Viral Meningitis[15].

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Cause	Key diagnostic test	Other potentially useful tests
Enteroviruses	CSF PCR	Throat and rectal swabs—culture, PCR
Herpes Simplex Virus (HSV)	CSF PCR	HSV type specific serology. Detection in genital lesions—PCR, culture, immunofluorescence, electron microscopy, Tzanck smear
Varicella Zoster Virus	CSF PCR	Detection in skin lesions—PCR, culture, immunofluorescence, electron microscopy, Tzanck smear
HIV	Serology	Serial IgG or combined IgG and antigen tests—HIV viral load (plasma, CSF)
Mumps Virus	Serology (serum, oral fluid)	PCR (throat swab, urine, EDTA blood, oral fluid)
Epstein-Barr Virus (EBV)	EBV specific serology, VCA IgM and IgG, EBNA IgG	CSF PCR. Monospot test

EBNA=Epstein-Barr nuclear antigen; PCR=polymerase chain reaction; VCA=viral capsule antigen; EDTA=ethylenediaminetetraacetic acid.

These tests offer an advantage over traditional culture methods because they are often more sensitive as well as quicker than the incubation times necessary for bacteria to grow, and because PCR tests exist for viruses as well as bacteria. PCR primers for most of the common bacterial pathogens also exist and can be used in addition with viral PCR to simultaneously test for viruses and bacteria[23].

1.5 REVIEW OF PREVIOUS STUDIES

Numerous studies have attempted to analyze CSF diagnostic values in healthy newborns. A summary of the authors, year the study was conducted, study population, and major findings can be seen in Table 4. A further description of these studies follows.

Table 4: A Summary of the Major Studies Involving the Analysis of CSF Constituents[5,11-14,24,25].

Author	Year	Population	Age Groupings/ Strata	CSF WBC (cells/mm^3)	CSF Glucose (mg/100mL)	CSF Protein (mg/100mL)
Samson[11]	1931	Infants < 6 months	0-2 weeks 2 wks-3mo 3-6 mo	3.33 3.00 1.33	No data	60.0 32.0 24.0
Widell[11]	1958	48 children <2 months	0-6 days 7-13 days 14-27 days 28-41 days 42-59 days	7.50 (avg for all groups)	No Data	80.9 70.4 53.9 46.5 34.8
Naidoo[12]	1968	135 infants < 24 hours		Polymorphs: 3.00 Lymphocytes: 2.00	51.0	63.0
Sarff *et al.*[5]	1976	117 infants	Term Preterm	8.20 9.00	81.0 74.0	90.0 115.0
Pappu *et al.*[24]	1982	24 infants < 32 days		11.00	No data	32.0-80.0
Portnoy and Olson[25]	1985	371 infants and children, birth to 10 years	< 6 weeks 6 wks-3mo 3-6 mo 6-12 mo >12 mo	3.37 2.92 1.88 2.63 1.94	No Data	No Data

Table 4: Continued

Author	Year	Population	Age Groupings/ Strata	CSF WBC (cells/mm^3)	CSF Glucose (mg/100mL)	CSF Protein (mg/100mL)
Bonadio *et al.*[21]	1992	75 infants < 8 weeks	0-4 weeks	11.00	46.0	84.0
			4-8 weeks	7.10	46.0	59.0
Ahmed *et al.*[14]	1996	108 infants < 30 days	1 week	15.30	45.9	80.8
			2 week	5.40	54.3	69.0
			3 week	7.70	46.8	59.8
			4 week	4.80	54.1	54.1

One of the first studies performed to examine the normal values of the CSF was published in 1931 by Samson. He investigated the WBC and protein levels in infants ages 0-2 weeks, 2 weeks-3 months, and 3-6 months[11]. Samson did not include the total number of infants studied; therefore it is uncertain what inclusion criteria he used. The results from Samson's work can be seen in Table 5. Samson's major contribution to the field was that he found that children ages 6 months and older have similar WBC counts as adults[11].

Table 5: Samson's Study of the Cerebrospinal Fluid in Infants[11].

Age	CSF WBC count (mean)	CSF Protein (mean)
0-2 weeks	3.33 cells/mm^3	60.0 mg/100mL
2 weeks – 3 months	3.00 cells/mm^3	32.0 mg/100mL
3-6 months	1.33cell/mm^3	24.0 mg/100mL

The next significant study was done by Widell in 1958. Widell also examined the CSF WBC and protein levels in infants. This study looked at 48 children under 1 year of age. No

children were included who had fever or any symptoms of central nervous system disease. All of the children had a lumbar puncture done between the second and third lumbar vertebrae[11]. According to the results, the greatest number of RBC in any of the punctures was 675 cells/mm^3, so this infers that none of the punctures was considered traumatic. Widell's study found that the number of WBCs in the CSF was elevated during the first three months of life, especially within the first two weeks, compared to the latter nine. The exact numerical results for results for the CSF WBC counts are not given by age in weeks, although it is stated that within the first two weeks of life, the number of CSF WBCs varied between 0 and 15 cells/mm^3, with a mean of 7.5 cells/mm^3 [11]. Widell's graph of CSF WBCs by age can be seen in Figure 8.

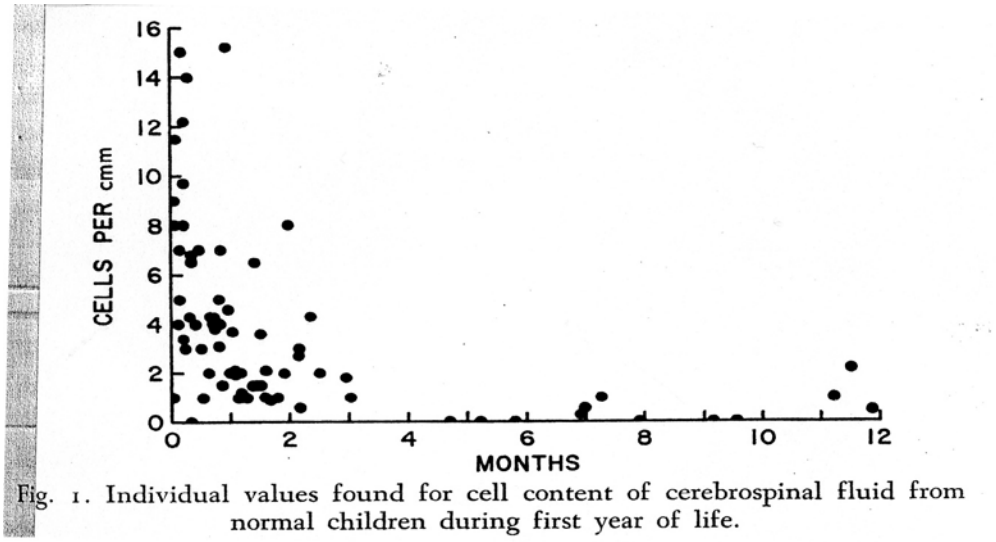

Fig. 1. Individual values found for cell content of cerebrospinal fluid from normal children during first year of life.

Figure 8: Widell's Investigation of CSF WBC in Infants During the First Year of Life[11]. Copyright Permissions Granted.

The protein levels were also seen be greater within the first three months of life as compared to the latter nine[11]. The figure from Widell's manuscript can be seen in Figure 9.

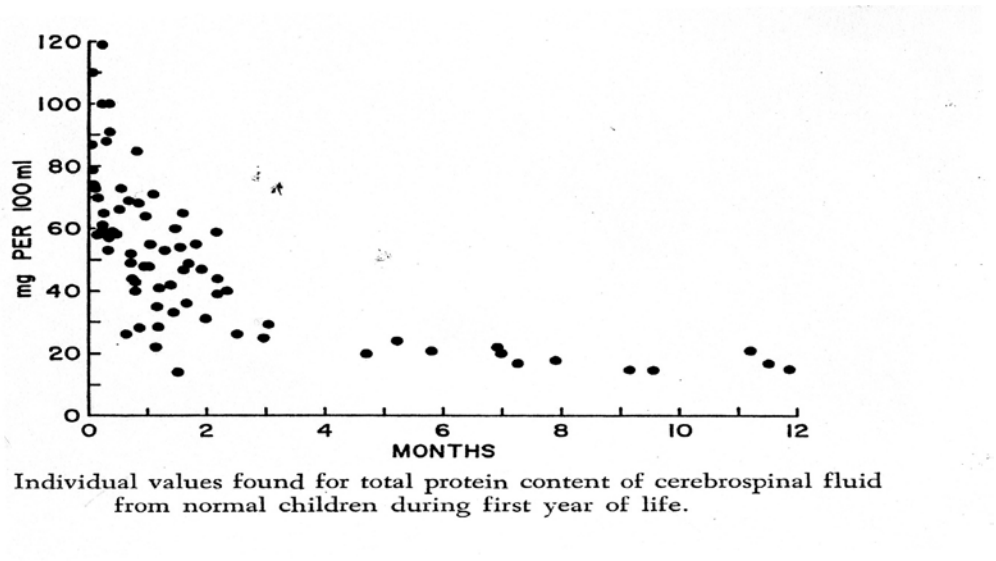

Figure 9: Widell's Distribution of Protein per 100mL of CSF[11].

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Table 6 summarizes the protein levels found by Widell for infants ages 0-6 days, 7-13 days, 14-27 days, 28-41 days, and 42-59 days.

Table 6: Widell's Study of Protein Levels in the CSF[11].

Age	Total Protein (mg/100mL)
0-6 days	80.9
7-13 days	70.4
14-27 days	53.9
28-41 days	46.5
42-59 days	34.8

Widell's study was followed in 1968 by Naidoo. In this study, Naidoo examined 135 newborn infants within the first 24 hours of life who were born at King Edward VII Hospital in Durban, South Africa. All healthy babies were included, and any who had "any obvious pathology were excluded from the study"[12]. Naidoo seems to have performed all the lumbar

punctures himself and he chose to perform the procedure with the children in a sitting position and by inserting the needle between the first and second lumbar space. A subgroup of 20 infants had repeat lumbar punctures performed at seven days of age to compare the newborn values with infants one week of age. Naidoo does not provide his results for WBC alone. Rather, he stratifies the results by polymorphonuclear cells and lymphocytes. The results of Naidoo's studies are seen in Table 7 for infants 1 day old and in Table 8 comparing 1 day olds and 7 day olds.

Table 7: Naidoo's Examination of CSF values within the first 24 hours of life[12].

Characteristic	Range	Mean	2 Standard Deviations
Red Blood Cells (cells/mm^3)	0-1070	9.00	0-884.00
Polymorphs (cells/mm^3)	0-70.00	3.00	0-27.00
Lymphocytes (cells/mm^3)	0-20.00	2.00	0-24.00
Proteins (mg/100mL)	32-240.00	63.0	27.0 - 144.0
Sugar (mg/100mL)	32-78.00	51.0	35.0 - 64.0

Table 8: Naidoo's Comparison of 20 Infants on Day 1 of Life and Day 7 of Life[12].

	Day 1		Day 7	
Characteristc	**Range**	**Mean**	**Range**	**Mean**
Red Blood Cells (cells/mm^3)	0-620.00	23.00	0-48.00	3.00
Polymorphs (cells/mm^3)	0-26.00	7.00	0-5.00	2.00
Lymphocytes (cells/mm^3)	0-16.00	5.00	0-4.00	1.00
Protein (mg/100mL)	40.0 - 148.0	73.0	27.0 – 65.0	47.0
Sugar (mg/100mL)	38.0 – 64.0	48.0	48.0 – 62.0	55.0

From Table 7, we can see that Naidoo did not exclude infants who had traumatic lumbar puncture. We can also see that the ranges for newborn infants are highly variable. Naidoo's data show that within seven days, the WBC and protein levels drop drastically, from 12 total cells/mm^3 to 3 cells/mm^3 for CSF WBC counts and from 73 mg/100mL to 47mg/100mL of protein. Glucose, however, remains nearly constant, at 48mg/100mL at age 1 day to 55 mg/100mL at age 7 days. Finally, Naidoo concludes, comparing his data to a cohort of infants ages 3 months to 1 year of age, that CSF values reach "normal" adult levels at age 3 months.[12] Besides Naidoo's inclusion of infants with traumatic lumbar punctures, his study is a solid study of infants ages 0-7 days. He does not consider infants any older than one week of age, however, even though he states that values approach "normal" values within 3 months of life. This study would have been more significant to the field if he had included more infants or continued

testing infants by week of age to get an accurate idea of the transitions of CSF WBC, protein, and glucose levels from birth to CSF "adulthood".

In 1976, Sarff *et al.* examined the differences between CSF in high risk infants with and without meningitis. For this study, 132 neonates born at Parkland Memorial Hospital in Dallas, Texas were included. These infants had lumbar punctures performed because they were at "high risk" for developing meningitis. Sarff *et al.* defined "high risk" as "unexplained jaundice, prematurity, prolonged rupture of membranes, chorioamionities, abruption placenta, maternal fever, and toxemia"[5]. The majority of the eligible infants were less than seven days old (100/117). The infants were divided into two groups – term and preterm, and then their CSF was analyzed for WBC count, protein, and glucose. The results for term vs. preterm infants can be seen in Table 9.

Table 9: Sarff *et al.*'s Comparison of High Risk Term and Preterm Infants[5].

Characteristic	**Term N=87**	**Preterm N=30**
White Blood Cells (cells/mm^3)		
Mean	8.20	9.00
2 Standard Deviations	0-22.40	0-25.4
Protein (mg/100mL)		
Mean	90.0	115.0
Glucose (mg/100mL)		
Mean	81.0	74.0

Sarff *et al.* found that for preterm infants there was a significant positive correlation of the WBC count values increasing throughout the first week of life. For term infants, however, there was no such correlation and levels tended to drop, although not to a significant level.[5] Although Sarff *et al.* state that they removed infants who had undergone a traumatic lumbar puncture, they state that the range of RBC in the CSF ranged from 0-46,000 cells/mm^3 in term infants and 0-39,000 cells/mm^3 for preterm infants. These values clearly show that infants who suffered a traumatic lumbar puncture were included in the analysis. As for protein and glucose, Sarff *et al.* state that there were no significant differences between these values between term and preterm infants[5]. Importantly, Sarff *et al.* also compared their values for "normal' neonates to a cohort of 119 infants with bacterial meningitis. They found that although there was some overlap between individual values, not one child with meningitis fell into the category of having "normal" values for CSF WBC, glucose, and protein levels[5]. This finding reinforces the idea that using these criteria as a predictor for meningitis can be beneficial, as long as all values are taken into consideration.

In 1982 Pappu *et al.* expanded upon the work of Sarff *et al.* and Naidoo and explore the differential composition of CSF WBC early in life. In this study, 24 normal birth weight infants between 1 and 32 days of age were analyzed for their CSF WBC differential. The infants included in this study were admitted to the hospital for being at a high risk for the development of meningitis. Infants were excluded if they had RBC counts >500cells/mm^3, and had positive blood or CSF cultures[24]. Although Pappu *et al.* gives a mean value for the WBC count, only a range is included for protein levels. The results from the study can be seen in Table 10.

Table 10: Pappu *et al.*'s Study of 24 Normal Birth Weight Infants Ages 1 to 32 days for CSF Total WBC and Protein[24].

	WBC Count (cells/mm^3)	Protein (mg/100mL)
Normal Birth Weight Infants (n= 24)	11.00 (1.00 – 38.00)	32.0 – 80.0

Pappu *et al*'s study had a relatively low number of patients and did not indicate what inclusion criteria were used to determine what was considered "high risk" for the development of meningitis. Despite these drawbacks, it is important to note that determining accurate measurements of WBC count and protein for infants was not the main objective of this study. However, the information collected by Pappu *et al.* aligns well with data found previously found in infant populations.

The next major study of CSF composition was a retrospective chart review conducted in 1985 by Portnoy and Olson. In this study, 371 infants and children were divided into five age categories : < 6 weeks, 6 weeks to 3 months, 3-6 months, 6-12 months, and >12 months. The youngest infant was approximately 1 day old whereas the oldest child was 10.5 years old. Infants were included in this study if they were "sick enough to warrant CSF examination, but who were found to have disease unrelated to CSF infection[25]". Infants were also included if they had suffered seizures prior to examination, in an attempt to determine whether infants with seizures require a different set of values than those without seizures. Infants were excluded if they had illnesses that were attributed to CSF infections of viral or bacterial nature. One of Portnoy's aims in this study was to characterize the distribution of WBC counts among age groups. Therefore, the means, standard deviations, and 25th, 50th, and 75th percentiles were calculated for each age group. The results can be seen in Table 11.

Table 11: Portnoy and Olson's Study on CSF Distributions Among Infants and Children[25].

Age	N	Mean WBC (cells/mm^3) (+/- standard deviation)	25%	50%	75%
<6 weeks	64	3.37 (+/- 3.40)	0.50	2.57	5.16
6 weeks-3 months	67	2.92 (+/- 2.86)	0.34	1.86	3.75
3-6 months	84	1.88 (+/- 2.01)	0.00	1.11	2.31
6-12 months	75	2.63 (+/- 2.45)	0.41	1.47	3.25
>12 months	81	1.94 (+/- 2.72)	0.00	0.68	1.82

From these data, we see that the highest variability is in infants < 6 months of age. As age increases, the variability in the CSF WBC values also decreases. We also see that the majority of infants <3 months do not have 0 cells/μL in their CSF, whereas it is more common in infants/children greater than 3 months of age.[25] This observation reinforces data previously discussed where CSF levels reached "normal" limits at between two and three months of age. In addition to these data, Portnoy and Olson examined CSF values in patients without seizures and patients with seizures. They found that for all age groups, there were no significant differences in WBC counts between patients with and without seizures[25]. From their data, Portnoy and Olson suggest that using the cutoffs presented from their study will ensure that few infants/children will be missed who have actually have bacterial/viral meningitis.[25] Portnoy and Olson's study was very beneficial to the field because it included infants who were actually

presenting with symptoms similar to those seen in meningitis. Also, it used a wide variety of age groups to characterize CSF values. It is our opinion, however, that the system used by Portnoy and Olson should be expanded to include age stratifications within the early time points. Because it appears as though variability is much lower after the 3 month time point, analyzing data from infants by week of age will provide needed information about the variation seen within the first few months of life in terms of CSF composition. Additionally, Portnoy and Olson do not state whether traumatic lumbar puncture was included and they do not analyze the CSF for glucose or protein. These shortcomings make this study an incomplete view on the characteristic values of the CSF in infants.

In 1989, Spanos *et al.* took a different approach to the problem of defining CSF characteristics. Instead of looking at normal values of CSF, they chose to look at CSF values in cases of bacterial and viral meningitis in order to determine the predictive values of CSF WBC, protein, glucose, and CSF-blood glucose ratio. For this study, they examined 422 patients who presented with bacterial or viral meningitis at Duke University Medical Center in Durham, NC between January 1969 and July 1980[4]. It is important to note that for this analysis they excluded all patients under 1 month of age. Using an advanced algorithm, they found that four independent measurements were able accurately to predict 55 cases of bacterial meningitis and 56 cases of viral meningitis. These measurements were total WBC count in the CSF, glucose, protein, and date of onset (past August 1).[4] Date of onset was taken into consideration because viral meningitis and some types of bacterial meningitis have higher rates of incidence in the summer months. Although this study excludes the majority of the study population with which we are concerned, it suggests that for more mature infant populations, the initial measurements of CSF can be predictive of bacterial and viral meningitis. More importantly, it also suggests

that no single criterion can accurately predict meningitis and that multiple factors need to be taken into consideration. More studies are needed on infant populations <1 month of age to ensure that these trends are similar across ages.

In 1992, Bonadio *et al.* sought to determine the reference values for CSF composition in infants 0-4 weeks and 4-8 weeks. Bonadio *et al.* justifies his use of 0-4 weeks and 4-8 weeks as his age strata because of the use of intramuscular ceftriaxone in the treatment of infants with meningitis ages 4-8 weeks. This drug is an antibiotic that can be used to target both gram positive and gram negative bacteria and is a drug of choice in treating infants with suspected meningitis.[13] In this study, they analyzed data from seventy five full-term infants who presented to the Children's Hospital of Wisconsin with a fever (>38.0°C). Infants were excluded from the study if they had received antibiotics prior to admission, had CSF samples that were indicative of traumatic lumbar puncture (>1000 RBC/mm^3), and had CSF gram stains and cultures that were positive for bacteria and viruses. Additionally, no infants were included in this study if they had seizures prior to being admitted to the hospital[13]. Bonadio *et al.* calculated the means, standard deviations, 95% confidence intervals, medians, and 90^{th} percentiles for CSF WBC counts. For protein and glucose levels, they calculated means and standard deviations. The results from this study can be seen in Table 12.

Table 12: Bonadio *et al.*'s Evaluation of Infants Ages 0-4 and 4-8 Weeks of Age for CSF WBC, Glucose, and Protein levels[13].

CSF Parameter	0-4 weeks n = 35	4-8 weeks n = 40	P values
Total WBC count (cell/mm^3)			
Mean (+/- standard deviation)	11.00 (+/- 10.43)	7.10 (+/- 9.23)	.0016*
95% confidence interval	7.35-14.57	4.27-9.99	
Median	8.50	4.50	
90th percentile	22.00	15.00	
Glucose (mg/100mL) (+/- standard deviation)	46.0 (+/- 10.3)	46.0 (+/- 10.1)	Not Significant
Protein (mg/100mL) (+/- standard deviation)	84.0 (+/- 45.1)	59.0 (+/- 25.3)	.0007**

* test performed using the Wilcoxin Rank Sum Test
**test performed using the student's t-test

As these data show, significant differences exist between infants aged 0-4 weeks and 4-8 weeks in terms of WBC and protein levels. Values were not significant for glucose levels, however. These data suggest that, for infants 0-4 weeks of age, 90% will have CSF WBC counts less than 22 cells/mm^3 and for infants 4-8 weeks, 90% will have values less than 15 cells/mm^3.[13] These values are vastly different from those seen in previous studies and can be attributed to better inclusion criteria. Overall, we believe the study design used by Bonadio *et al.* is sound, and, in fact, the majority of the criteria used to identify study populations will be used in our study as well. One improvement that we argue is necessary is to further stratify the infants by week of age. We believe that, if differences exist between infants aged 0-4 weeks and 4-8 weeks, there may also be significant differences in infants by week of age.

The next study on normal CSF values in infants was done in 1996 by Ahmed e*t al*. This study used the recently advanced enterovirus PCR tests to help make inclusion criteria more stringent and precise. This study used two prospective studies from June, 1993 to October, 1994 in Children's Medical Center of Dallas. The inclusion criteria included full term infants who had "an atraumatic lumbar puncture (<1000 RBC/mm^3), no prior antibiotic therapy, sterile blood, CSF, and urine bacterial cultures, negative CSF viral culture, and negative CSF PCR for enteroviruses"[14]. CSF total WBC counts were determined using the Neubauer counting chamber and protein and glucose concentrations were determined using standard methods. Together, 207 infants younger than 30 days were selected, and of these 108 met all inclusion criteria. The results can be seen for week of age in Table 13.

Table 13: Ahmed *et al.*'s Study of Infants Ages 0-30 Days for CSF WBC, Protein, and Glucose levels[14].

CSF Parameter	1 week 0-7 days N=17	2 weeks 8-14 Days N=33	3 weeks 15-21 days N=25	4 weeks 22-30 days N=33
WBC (cells/mm^3)				
Mean (+/- standard deviation)	15.30 (+/- 30.30)	5.40 (+/- 4.40)	7.70 (+/-12.10)	4.80 (+/-3.40)
95% confidence interval	12.00 - 18.10	4.60 - 6.10	6.30 - 9.10	4.10 - 5.40
Median	6.00	6.00	4.00	4.00
Range	0-13.00	0-18.00	0-62.00	0-18.00
90th percentile	18.00	10.00	12.50	8.00
Protein (mg/dL) (+/- standard deviation)	80.8 (+/- 30.8)	69.0 (+/-22.6)	59.8 (+/- 23.4)	54.1 (+/-16.2)
Glucose (mg/dL) (+/- standard deviation)	45.9 (+/- 7.5)	54.3 (+/- 17.0)	46.8 (+/- 8.8)	54.1 (+/- 16.2)

This study showed that significant differences existed between infants for WBC counts based on week of age. Protein levels were significantly higher in the first two weeks of life than in the 3rd and 4th weeks[14]. Despite these findings, we feel that this study was limited by sample size. Their 1st week cohort only had 16 patients, whereas the others had nearly double this. This

is not balanced in terms of numbers in the other categories and although the difference is not large enough to affect the power of the study and the ability for statistical differences to be determined, we feel a larger sample size should be used. By using similar inclusion criteria and a larger sample size, we will be better able to determine whether real differences exist in infants by week of age for CSF WBC, glucose, and protein levels. Additionally, we feel as though combining Bonadio *et al.*'s findings with this study would be beneficial. That is, stratifying infants less than two months old by their age in weeks and determining the values will be the most best way to generate appropriate values for normal CSF WBC, protein, and glucose.

These studies, taken over nearly a 70 year period, have shown that the question of addressing normal CSF values for infants is a difficult one. Besides age, other factors, including disease status, lumbar puncture success, antibiotic treatment, and gestational outcome (full term or preterm), need to be included when choosing patients to study. We believe that these studies have provided excellent background for our current study and have shown us what is important to include when choosing our own study population. We feel as that we can improve on these studies by adopting stringent inclusion criteria and by having a large sample size for all age groups.

2.0 MATERIALS AND METHODS

2.1 CHART SELECTION AND REVIEW

For this study, three separate cohorts were used from three different time periods ranging from 1995 to 2008. The study designs were approved individually by the University of Pittsburgh Institutional Review Board (IRB). Dates, reference names, number of observations, and analysis variables that were collected can be seen in Table 14.

Table 14: Summary Characteristics of the Cohorts Used for this Analysis.

Date Collected	Date Observed	Reference Name	Number of Observations	Analysis Variables
1999	March 1995 – February 1996	1995 cohort	536	CSF WBC CSF RBC
2003	December 2001-December 2002	2002 cohort	446	CSF WBC CSF RBC CSF Glucose CSF Protein
2009	January 2008 – February 2009	2008 cohort	415	CSF WBC CSF RBC CSF Glucose CSF Protein

The inclusion of three cohorts of infants is to determine if there are differences in the CSF constituent characteristics throughout time and to examine if significant changes in these values exist depending on birth cohort. Cases were identified using a retrospective chart review. For the first two cohorts, a paper chart review was performed using an honest broker system to de-identify patient information for analysis. For the third cohort, records were selected using an electronic database that automatically excludes identifying information and includes only factors necessary for analysis. Potential cases were identified as infants who presented to Children's Hospital of Pittsburgh's emergency room with a fever and other generalized symptoms of possible meningitis. Infants who had a lumbar puncture performed in the emergency room or 24 hours after admittance to the hospital were eligible to be screened for inclusion in this study. Infants were excluded from the study if they had a positive CSF bacterial culture, a positive CSF viral culture, a positive CSF viral PCR, the presence of a ventriculoperitoneal shunt, recent neurosurgery, had been on antibiotics prior to or at the time of the lumbar puncture, were born prematurely (< 36 weeks gestation), or had had seizures prior to presentation in the emergency room. Infants were included if they had a CSF RBC count < 1000 cells/mm^3, a CSF WBC count <100 cells/mm^3, and were younger than 60 days (or 8 weeks) of age. Additionally, for the second and third cohort, cases were included only if they had complete documentation of the CSF glucose and CSF protein along with the CSF WBC count.

The 1995 cohort study design was approved by the University of Pittsburgh IRB, study number CHP 02-120. A physical chart review identified 536 infants between March of 1995 and February of 1996 who had undergone a lumbar puncture upon presentation to the Emergency room of Children's Hospital of Pittsburgh. Unlike the other two cohorts, only CSF WBC and CSF RBC data were collected. CSF glucose and CSF protein were not included in this

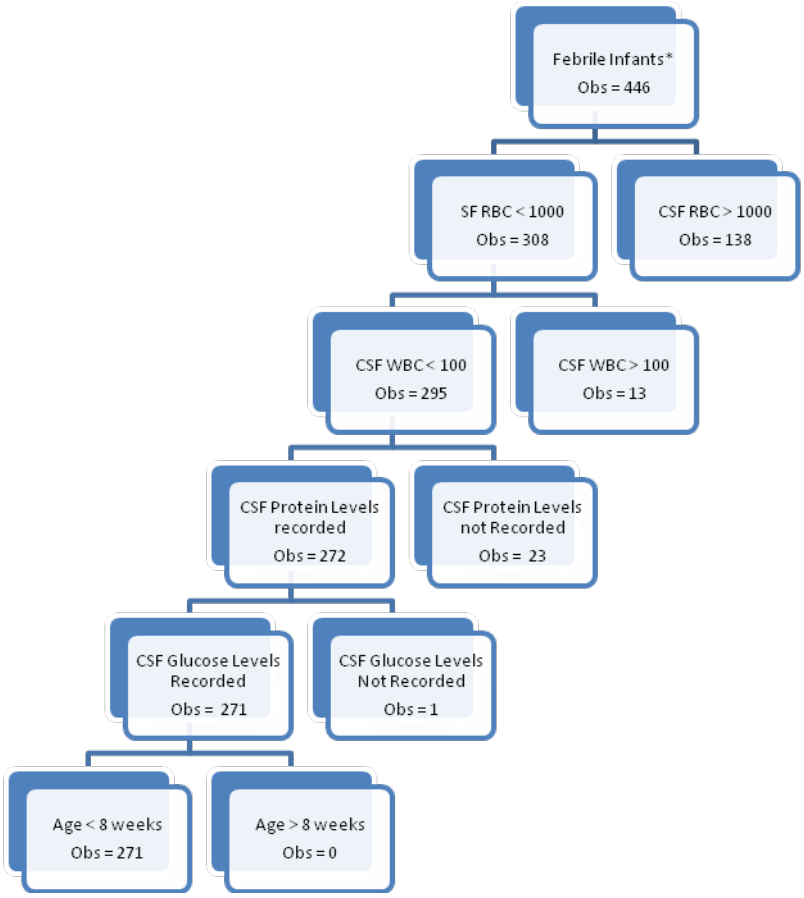
collection. From the charts a de-identified database was constructed which included the date of the lumbar puncture, birth date, CSF WBC counts, CSF RBC counts, and CSF culture results. The sex of the patient was not recorded in this chart review. Because we do not suspect that gender affects CSF WBC, glucose, or protein levels in infancy, we were not concerned with this lack of information. All 536 infants included in this study met the criteria outlined above.

The 2002 cohort study design was approved by the University of Pittsburgh IRB, study number 04051761. A physical chart review identified 446 infants who presented between December 2001 and December 2002 eligible for screening. From these files, a de-identified database was created that included the information described in Table 15.

Table 15: Data Collected from 446 Patient Charts from April 2003 to April 2005.

Data Collected from Patient Records in Cohort 2	
Birth date	Date of Lumbar Puncture
Age in Days	Sex
Gestational Age	Reason for Lumbar puncture
Temperature in the ER	Previous antibiotic Therapy
CSF WBC	CSF RBC
CSF glucose	CSF protein
Peripheral WBC	Peripheral Glucose
Gram Stain Result – Bacteria	Bacterial Culture Result of CSF
Blood Culture Result	Urine Culture Result
Viral CSF culture result	Viral PCR results
Final Diagnosis on ER Chart	Final Diagnosis on Hospital Chart

These 446 infants were included for statistical analysis if they met the criteria outlined previously. Following application of these criteria, 271 infants remained for statistical analysis. A detailed schematic of infants included for statistical analysis can be seen in Figure 10.

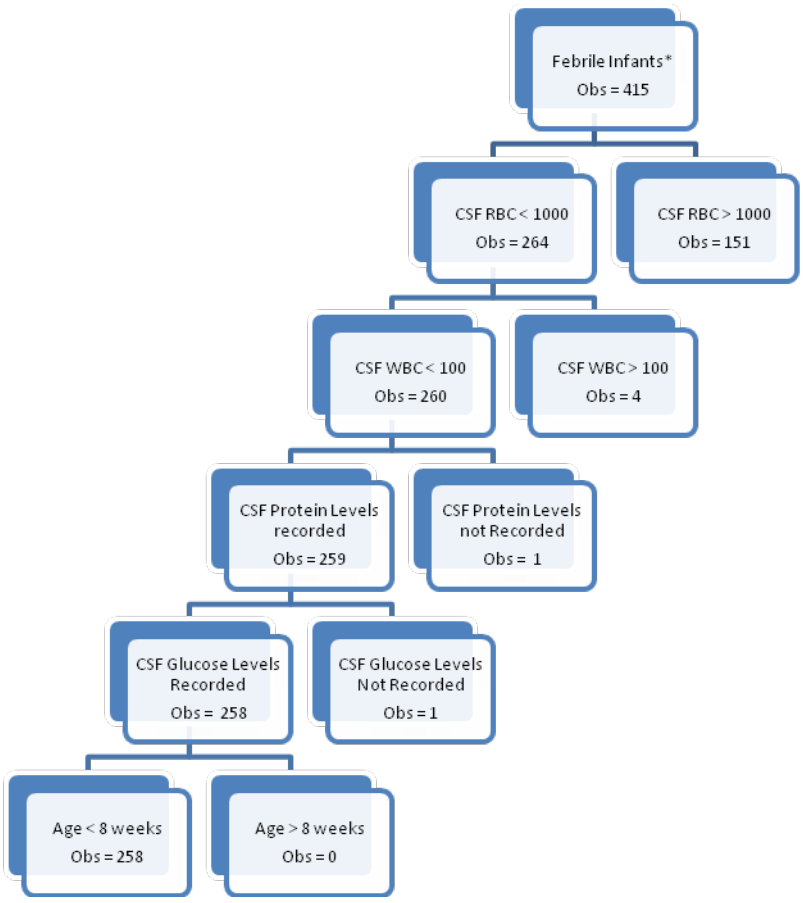


*These infants were those who met inclusion and exclusion criteria for further study.

Figure 10: A Flow Chart of Infants Selected from December 2001 to December 2002.

The 2008 cohort was collected in March 2009 using Cerner electronic medical records of patients who were less than 60 days of age who presented at Children's Hospital between January 2008 and February 2009 and met the inclusion and exclusion criteria. This study was

approved by the University of Pittsburgh IRB, study number PRO08090308. Data collected for this cohort included age in days at the time of lumbar puncture, time from entry into ER or hospital to lumbar puncture, CSF WBC, CSF RBC, CSF protein, CSF glucose, and CSF culture results. Again, sex was not included in this chart review. Charts were checked for positive CSF bacterial or viral cultures or PCRs. Following collection, charts were checked by hand to see whether CSF pleocytosis existed (CSF WBC > 10cells/mm^3) to ensure that all infants were being included only in the study if they met the criteria described previously. The third cohort had 415 observations. A flow chart can be seen in Figure 11 to show the progression of criteria used to identify 258 cases for statistical analysis.

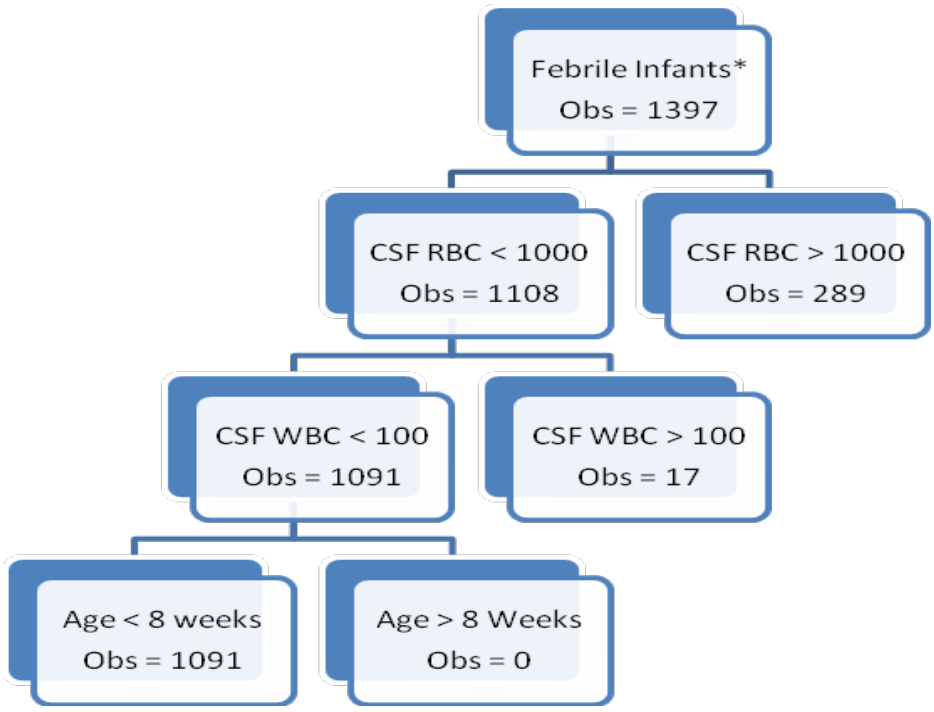


*These infants were those who met inclusion and exclusion criteria for further study.

Figure 11: A Flow Chart of Infants Selected from January 2008 to February 2009.

Finally, the datasets were combined to determine if the variance between populations was low enough so that the observations could be combined and one set of data values could be made to describe the normal values of CSF WBC, CSF glucose, and CSF protein for infants. When combined, the datasets were screened using the same inclusion and exclusion criteria as the individual cohorts, except observations were not discarded if CSF protein or CSF glucose were absent, as the entire 1995 dataset lacks these measurements. That means that there are 26 additional observations in the combined dataset that were not included for analysis in the

individual analysis. Combining the datasets led to 1,397 infants. Following the application of inclusion and exclusion criteria, 1,019 infants were available for statistical examination. A flow chart of the cases included can be seen in Figure 12.



*These infants were those who met inclusion and exclusion criteria for further study.

Figure 12: A flow chart of infants combined from the 1995, 2002, and 2008 cohort.

2.2 STATISTICAL ANALYSIS

All data were analyzed using SAS Version 9 or Stata10.1. To determine if it was appropriate to pool the observations from the three cohorts, the variance between the cohorts needed to be analyzed to determine if it was the same for all cohorts. To start, data were stratified by age and gender (for the 2002 cohort) to make sure that no significant differences existed between the strata. The data were assessed for normality in respect to the major variables of interest (CSF WBC, glucose, and protein levels) using histograms and Kolmogorov-Smirnov tests for normality. If the data were normally distributed, differences between cohorts by age in weeks were assessed using Analysis of Variance (ANOVA). If the data were found not to be normally distributed, log-transformations were attempted to normalize the values. If the data were not normally distributed following transformation, a Kruskal-Wallis test was run on the data to determine whether significant differences existed between the variables of interest (CSF WBC, CSF glucose, and CSF protein) and age in weeks[26]. To compensate for the number of tests being done repeatedly, a Bonferroni adjustment was applied to these p values to determine significant differences[26]. In our analysis, we assumed an alpha of .05 and divided by 9 (one for each of our week stratifications) to give a Bonferroni adjusted critical p-value of .0056. If we found no significant differences, we assumed that it was possible to pool the observations and generate one set of means and confidence intervals for CSF WBC, protein, and glucose. These values were generated using the methods described above for the individual cohorts. If significant differences were found, then each cohort was analyzed independently.

If the observations were pooled, then the three questions being addressed by our study were considered for the entire cohort, and not for the individual cohorts. If the cohorts were not

pooled for any of the variables being assessed, then the same procedures were carried out as for the pooled variables, except each cohort was considered individually.

To determine whether there was a change in CSF WBC, glucose, and protein levels by age in weeks, locally weighted scatterplot smoothing (LOWESS) plots were constructed for each variable to visually assess differences across time[27]. If the cohort was normally distributed, then ANOVA was used to formally assess differences between the variables by age in weeks. If the cohort was not normally distributed, then the Kruskal-Wallis test was used with week as a grouping variable to seek differences by age in weeks.

If significant differences were found for variables by age in weeks, means for each week were calculated using standard methods. Ninety-five percent confidence intervals for the mean were generated using standard methods if the variables were normally distributed. If the variables were not normally distributed, the 95% confidence intervals were calculated using the non-parametric bootstrap method. The bootstrap method does not require that the data follow a normal distribution, and uses a series of samples with replacement from the original sample to estimate the confidence intervals surrounding the mean[28]. Ten-thousand samples were used to generate 95% confidence intervals in this analysis. If significant differences were not found to exist for variables by age in weeks, then the variables were re-categorized by age in months and reassessed using the methods described earlier. If no differences existed within these divisions, then means and 95% confidence intervals were calculated for the entire cohort as one age group using the methods described previously.

To generate a "normal" range of values that can be expected for CSF WBC, protein, and glucose, the median and the 10^{th} and 90^{th} percentiles were calculated using standard methods[26]. If differences were found between age groupings, each age category had its own median and

percentiles calculated. If no differences were found, the median and percentiles were calculated for that variable for the entire cohort.

3.0 RESULTS

3.1 DESCRIPTION OF COHORTS

As previously stated, three cohorts were considered for analysis. To observe the relative frequencies of the observations, the data were stratified by age in weeks. The number of patients in each cohort, the relative frequencies, medians, and ranges for CSF WBC, glucose, and protein for each age in weeks can be seen in Table 16. To determine age in weeks, the age in days (date of lumbar puncture – date of birth) was divided by seven. All infants were less than 60 days of age, and any infants greater than 56 days old (8 weeks) were not included in the analysis. Less than seven days was denoted as 0-1 week, 7-14 days as 1 week, 14-21 days as 2 weeks, etc.

Table 16: Descriptive Characteristics of the 1995, 2002, and 2008 Cohorts.

	Age (weeks)	**0-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1995	**N**	64	52	46	83	76	74	77	56	8
	Percentage	11.94	9.70	8.58	15.49	14.18	13.81	14.37	10.45	1.49
	CSF WBC* Median Range	4.5 0-33	3.00 0-20	3.00 0-23	3.00 0-33	3.00 0-44	2.00 0-14	1.00 0-22	2.00 0-33	1.50 0-3
2002	**N**	37	28	25	37	38	36	33	27	10
	Percentage	13.65	10.33	9.23	13.65	14.02	13.28	12.18	9.96	3. 69
	CSF WBC* Median Range	7.00 0-69	2.50 0-20	3.00 0-50	2.00 0-33	2.00 0-96	1.00 0-93	2.00 0-50	2.00 0-22	2.00 0-10
	CSF glucose** Median Range	49.00 32-104	51.5 36-80	49.0 33-64	2.00 42-77	49.5 40-137	53.0 39-81	52.00 41-88	52.00 41-88	52.5 41-70
	CSF protein** Median range	96.0 55-219	76.0 5-124	67.0 31-160	68.0 34-99	56.0 35-87	60.0 24-110	47.0 24-91	53.0 30-234	59.0 20-132
2008	**N**	17	21	36	21	36	36	44	29	18
	Percentage	6.59	8.14	13.95	8.14	13.95	13.95	17.05	11.24	6.98
	CSF WBC* Median Range	4.00 0-9	3.00 0-10	2.00 0-15	2.5 0-12	2.0 0-15	2.0 0-10	2.0 0-9	2.0 0-24	2.0 0-7

Table 16: Continued

CSF glucose**									
Median	45.0	47.0	44.0	43.0	46.5	44.5	47.0	46.0	45.5
Range	34-65	29-113	32-81	33-78	22-76	34-87	0-125	37-71	36-105
CSF protein**									
Median	105.0	74.0	63.5	71.0	66.5	51.00	49.5	59.00	47.0
range	44-185	52-120	37-118	41-275	30-126	31-140	24-127	29.0-134	26-84

***CSF WBC values are in cells/mm^3**

****CSF glucose and CSF protein are in mg/100mL**

The 1995 cohort contained 536 infants and was relatively evenly distributed in terms of weeks, except for the 8 week age category. This discrepancy in observations for the 8 week old category will affect the power for comparisons involving this group, and thus the associations may not be as strong as for the other week categories.

Once the 2002 cohort was modified to include our inclusion criteria, there were 271 observations left in our data set (Figure 9). Before analysis could begin, we wanted to observe the relative frequencies of our data in terms of gender and age. The 2002 data set had 160 males and 111 females, or 59.04% and 40.96% respectively. Although there are more males than females in this dataset, no data have been found in the literature that suggest that CSF WBC, glucose, and protein levels differ among newborn males and females. Next, the dataset was stratified by age in weeks to determine whether any major differences existed between the strata. As seen in the 1995 cohort, the 2002 cohort has fairly similar frequencies of patients across the week categories, except for the week 8 range. The other week categories have between 25 and 40

infants in each group. The 8 week range has only 10 infants. As previously discussed, this can affect the power of comparisons involving this stratum.

The 2008 dataset had 258 observations and included data for CSF WBC, CSF glucose, and CSF protein. To start, the data were stratified by week to determine the relative distribution by age in weeks for the infants. Unlike the 1995 and the 2002 cohorts, the 2008 dataset had a relatively limited number of observations in the first and eighth weeks. The majority of the values were seen in weeks 2-6. The stratum sizes ranged from 17 to 44 per week.

3.2 COMBINING THE COHORTS

Before analysis was done to see whether the values varied significantly for CSF WBC, glucose, and protein between the age stratifications, the normality of the CSF WBC variables was assessed. Normality is a key factor when choosing which statistical analyses to perform to ascertain significance. A histogram of the each variable's distribution was made for each cohort with a normal curve superimposed to help visually assess normality. Additionally, a formal normality test, the Kolmogorov-Smirnov test, was run. The Kolmogorov-Smirnov test was chosen for this analysis because it is appropriate for larger sample sizes[26]. All of the variables were found not to be normally distributed as determined by this formal test (p value <0.05). The histograms for each variable can be seen in Figures 13-15.

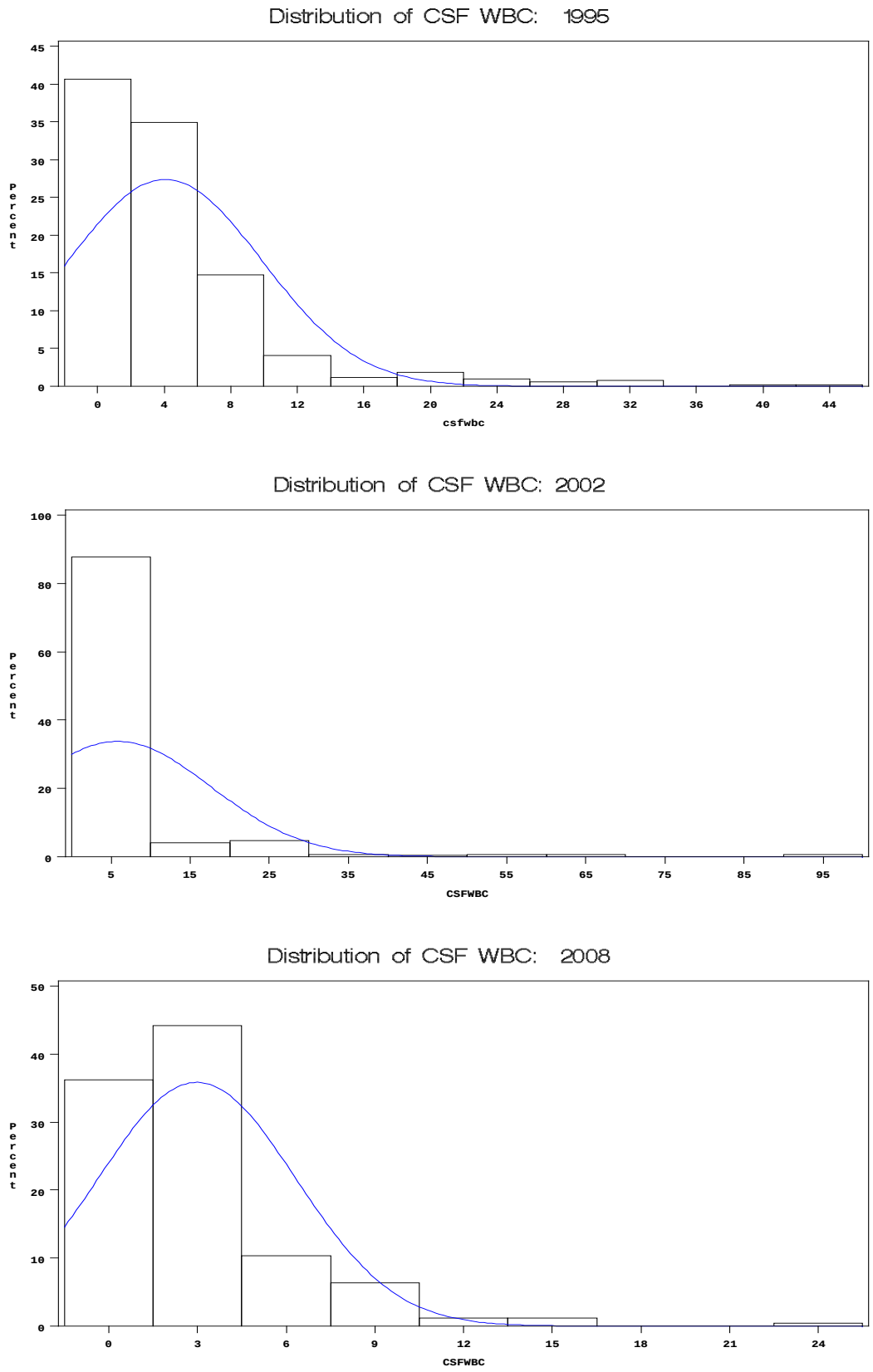


Figure 13: Distributions of CSF WBC for the 1995, 2002, and 2008 Cohorts.

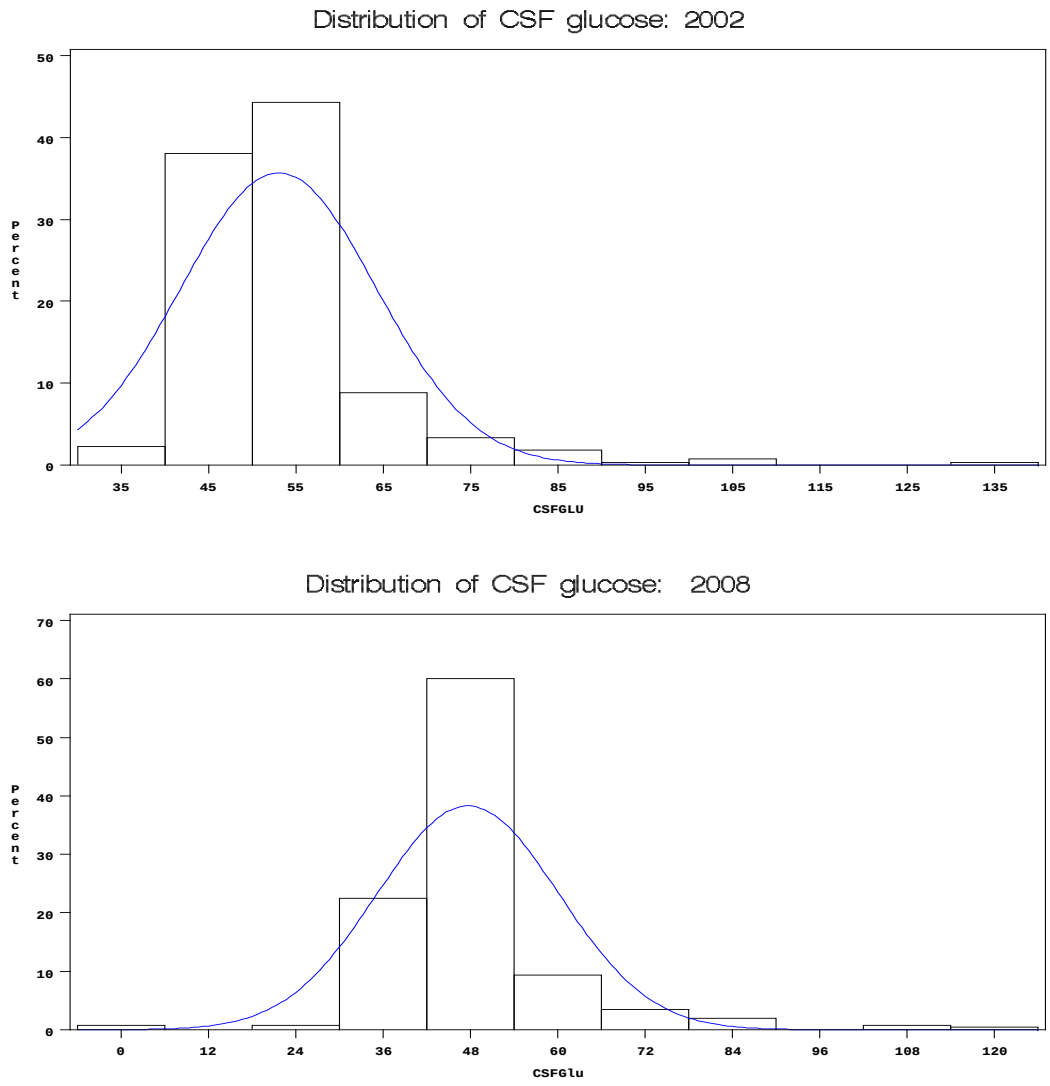


Figure 14: Distribution of CSF Glucose for the 2002 and 2008 Cohorts.

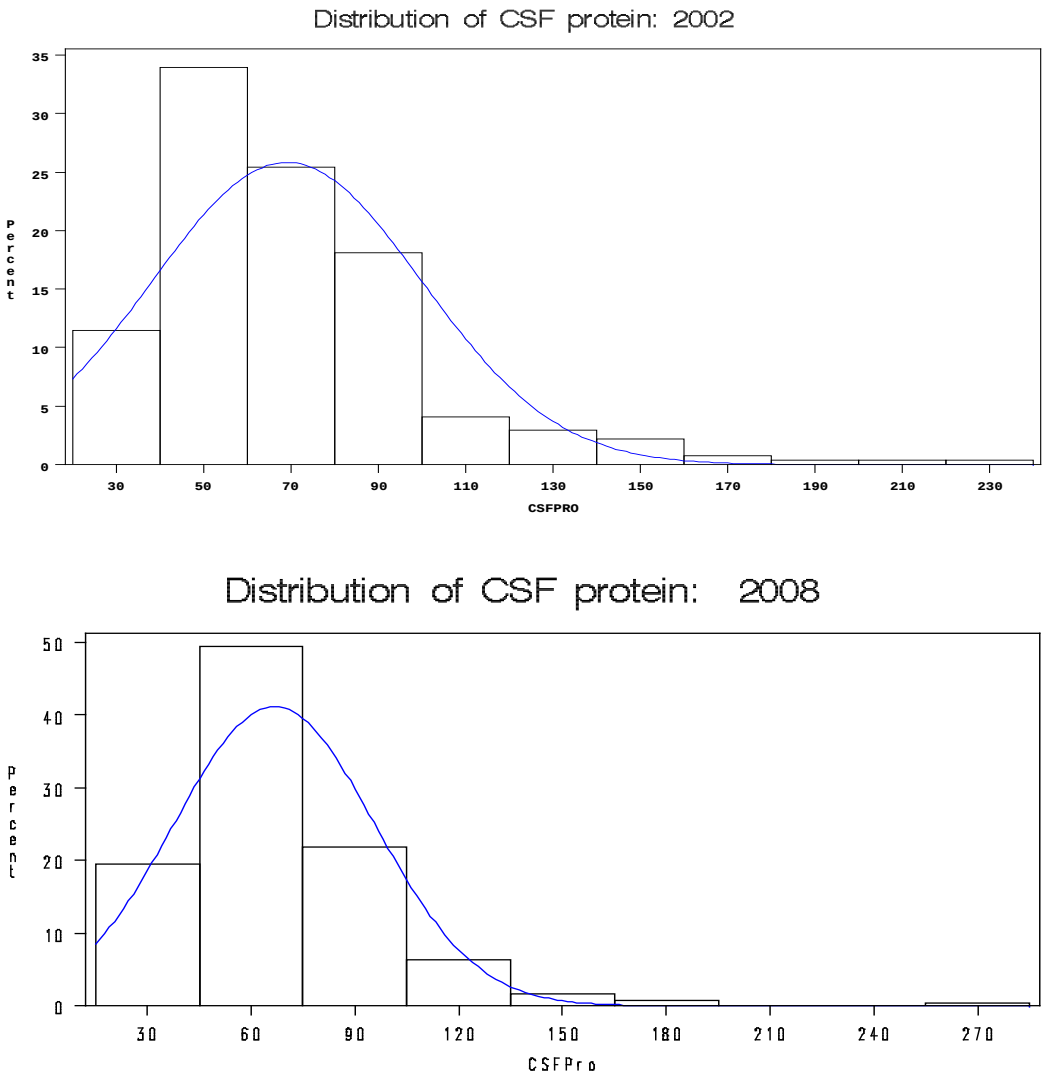


Figure 15: Distribution of CSF Protein for the 2002 and 2008 Cohorts.

To transform the variables in an attempt them follow a normal distribution, the log of each variable was calculated. Because Log(0) is an undefined number, the transformations were calculated using the following equations:

$$\text{LogWBC} = \text{Log}_{10}\,(\text{CSF WBC} + 1)$$

$$\text{LogGlu} = \text{Log}_{10}\,(\text{CSF Glucose} + 1)$$

$$\text{LogPro} = \text{Log}_{10}\,(\text{CSF Protein} + 1)$$

Once the transformations were completed, normality was reassessed using the same procedures as used on the untransformed variable. Following transformation, none of the variables passed the test for normality except for CSF protein in the 2002 cohort (Kolmogorov-Smirnov p-value >0.150). Other transformations were attempted in addition to the Log_{10} transformation, but none were successful in making the variable normally distributed. Because no transformation could make all the variables normally distributed, it was decided that non-parametric measures would be used to analyze the dataset.

When the datasets were combined and the inclusion criteria were applied to the observations, there were 1,091 infants available for statistical analysis. Because the 1995, 2002, and 2008 datasets had data for CSF WBC, all 1,091 infants were included for analysis for this variable. But because the 1995 dataset did not include data for CSF glucose or CSF protein, only the cases from the 2002 and 2008 dataset were included for this analysis. This left 530 infants available for analysis for the CSF glucose and the CSF protein variables (after removing all observations which did not have CSF glucose and protein observations in the 2002 and 2008 cohorts). The age distributions of the infants were calculated and the results of this distribution can be seen in Table 17. Each week category has a similar number of observations, except for the week 8 category, which had significantly fewer observations. This trend was seen for the

1995, 2002, and 2008 individual datasets as well, so it is no surprise that it persists when the sets are combined. This low number of observations can have effects on the power of the results for analysis on the 8-week old infants.

Table 17: Age Distribution of the Combined Cohorts.

Age (weeks)	**0-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Number CSF-WBC analysis	120	104	109	141	156	150	161	114	36
Percentage	11.0	9.53	9.99	12.92	14.30	13.75	14.76	10.45	3.30
Number CSF Glucose and Protein Analysis	54	49	61	58	74	72	77	56	29
Percentage	10.21	9.26	11.53	10.96	13.99	13.61	14.56	10.59	5.29

In order to determine whether the data from the three cohorts could be combined for analysis to generate a single set of values, non-parametric ANOVA using the Kruskal-Wallis test and a Bonferroni adjustment were used. We wanted to determine whether there are significant differences between the values for CSF WBC between the 1995, 2002, and 2008 datasets and between the values for CSF glucose and protein for the 2002 and 2008 datasets. Because our data are not normally distributed, we cannot use a standard regression approach to determine this. The data were analyzed using a Kruskal-Wallis test for each week to see whether the data

values could be pooled for the three time periods. Because there are 9 week categories which we are concerned about pooling, we will repeat each of these tests 9 times for CSF WBC, glucose, and protein. This necessitates the use of an adjusted alpha. To maintain an alpha of 0.05 across the collection of tests, we divided our alpha by nine to determine what is statistically significant. Our new p-value following this adjustment is 0.0056. If the p-values for each test are greater than this value, we can assume that no differences exist between the three cohort time periods and they can be pooled to generate a single range of values for the variable.

The results of the Bonferroni-adjusted Kruskal-Wallis test for CSF WBC values can be seen in Table 18. We find that no significant differences exist between the cohorts for each week. This means that we are able to generate a single set of values that accurately characterize CSF WBC count in infants by age in weeks.

Table 18: Kruskal Wallis using the Bonferroni Adjustment for CSF WBC Values Across the 1995, 2002, and 2008 Cohorts.

Week	Chi-Square Statistic	P-value
0-1	3.778	0.153
1	0.039	0.981
2	1.425	0.491
3	0.796	0.672
4	1.688	0.430
5	0.244	0.885
6	0.595	0.743
7	0.424	0.809
8	1.261	0.532

The results for the Bonferroni adjusted Kruskal-Wallis test for CSF glucose differences among all cohorts can be seen in Table 19. Unlike the CSF WBC, we do find some significant differences among the observations for this variable. Significant differences existed between the 2002 and 2008 cohorts for weeks 3, 5, and 6. Because of these differences, we will not pool the observations for CSF glucose.

Table 19: Kruskal Wallis using the Bonferroni Adjustment for CSF Glucose Values Across the 2002, and 2008 Cohorts.

Week	Chi-Square Statistic	P-value
0-1	3.105	0.078
1	5.536	0.0184
2	2.447	0.118
3	14.436	<0.0001*
4	5.946	0.0148
5	24.693	<0.0001*
6	12.8515	0.0003*
7	7.5595	0.006
8	2.365	0.124

* = significant results, $p<0.0056$

The results of the Kruskal-Wallis Bonferroni adjusted test for CSF protein for the combined dataset can be seen in Table 20. Similarly to the CSF WBC, there exist no statistically significant differences between CSF protein values among the 2002 and 2008 cohorts. This indicates that these observations can be pooled to generate a single set of values for CSF protein.

Table 20: Kruskal Wallis using the Bonferroni Ajustment for CSF Protein Values Across the 2002, and 2008 Cohorts.

Week	Chi-Square Statistic	P-value
0-1	0.083	0.773
1	0.7342	0.3915
2	0.560	0.454
3	0.2435	0.6217
4	5.2440	0.0220
5	0.8793	0.3484
6	1.5441	0.2140
7	0.0638	0.8805
8	0.8315	0.3618

These tests tell us that we will be able to pool the values for CSF WBC and CSF protein, but not for CSF glucose.

3.3 DETERMINING WHETHER DIFFERENCES EXIST FOR CSF WBC, CSF GLUCOSE, AND CSF PROTEIN BY AGE IN WEEKS

The first question we wanted to answer with this analysis was whether differences exist among the variables as infants' age increases by weeks. In order to address this question, LOWESS curves were made to visually analyze the trends through time. The LOWESS function calculates the means for a set of values in a window across the horizontal axis. Depending on the width of the window selected, the line can fit all of the data points (sense all the noise in a sample) or can generate a linear relationship between the data points. We used a window of 0.6 to generate a

smooth curve that describes the best relationship between our variables. These plots can be seen in Figures 16, 17, and 18.

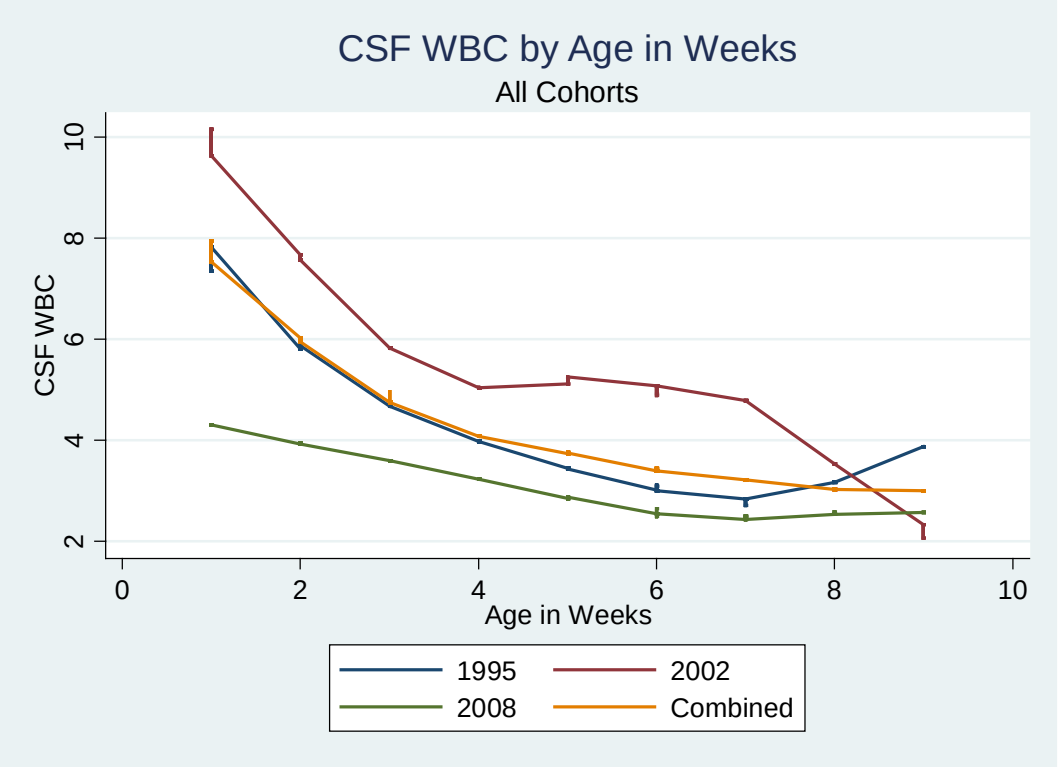


Figure 16: LOWESS Smooth Curve of CSF WBC for the 1995, 2002, 2008, and Combined Cohorts.

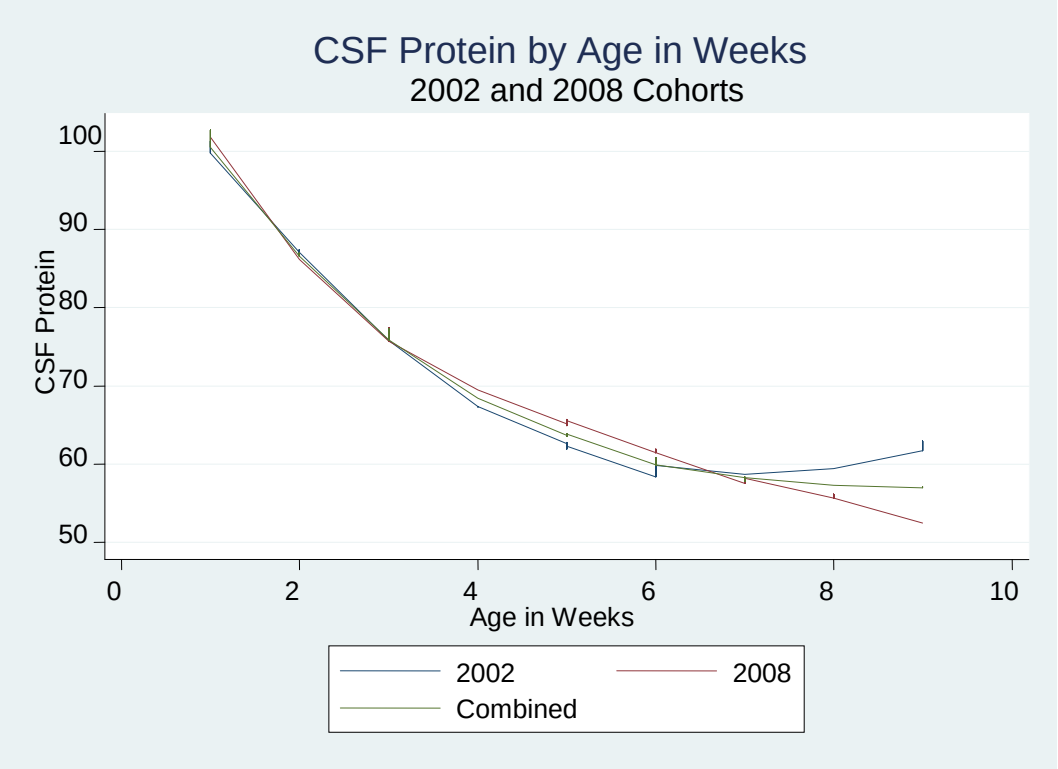
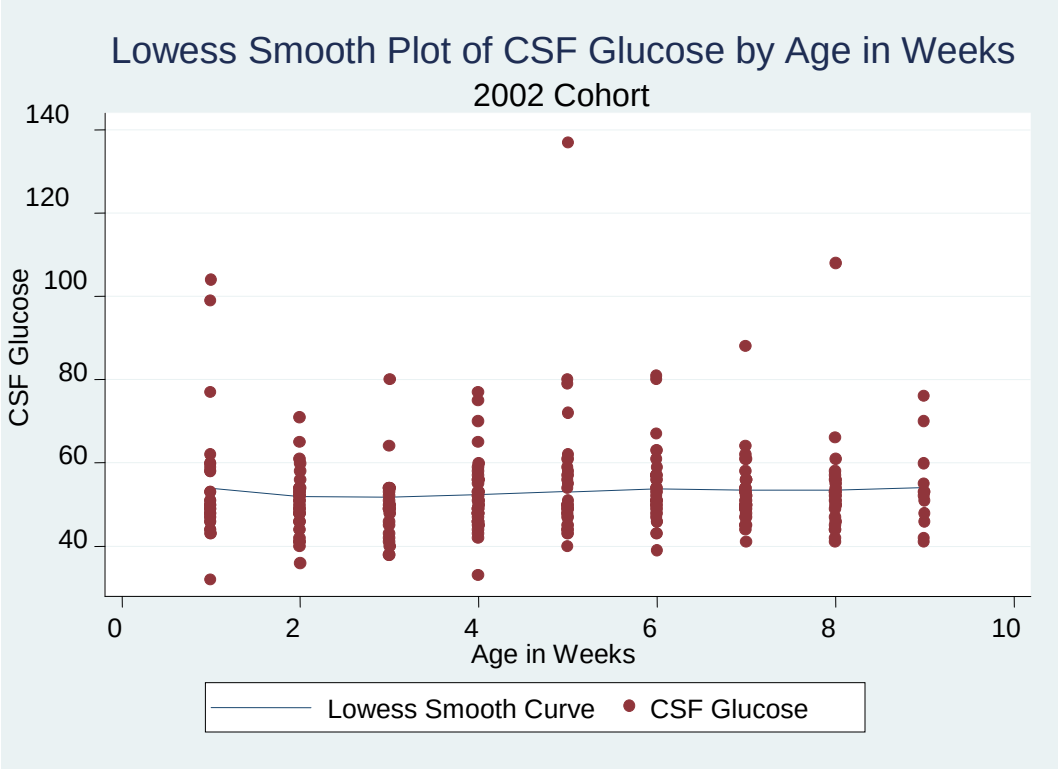


Figure 17: LOWESS Smooth Curve of CSF Protein for the 2002, 2008, and Combined Cohorts.



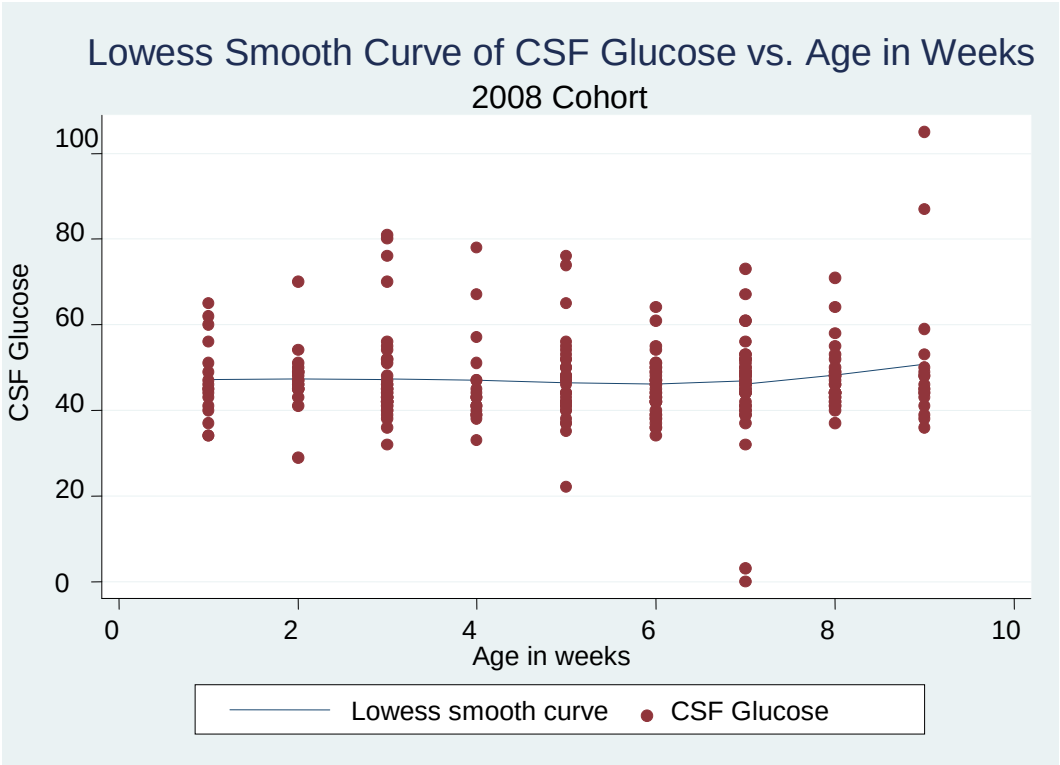


Figure 18: LOWESS Smooth Curve of CSF Glucose for the 2002 and 2008 Cohorts.

From these plots, we can see that for CSF WBC and protein, a decreasing trend does seem to be present that corresponds with increasing age. The values for CSF WBC and protein appear to be highest at birth and then decrease in a relatively consistent manner as age in weeks increases. For CSF glucose, however, we do not see this trend. In both the 2002 and 2008 cohorts, it appears as though the CSF glucose counts are relatively constant as age increases.

In order to formally address these obesrvations, non-parametric Kruskal-Wallis ANOVA tests were used on the pooled observations for CSF WBC and protein and on the individual cohorts for CSF glucose to test for differences between age groups. For CSF WBC, we found a chi-square test statistic of 92.077 with a p-value <0.0001. This indicates that significant differences exist for CSF WBC within the cohort among our age categories and confirms that the temporal trend seen visually in the LOWESS plot is significant. For CSF protein, the Kruskal-Wallis test produced a Chi-Square statistic of 118.8440 and a p-value <0.0001. This indicates that significant differences exist among the age in weeks for CSF protein and confirms the trend seen in the LOWESS plot. These findings indicate that it is appropriate to generate unique means, 95% confidence intervals, and expected ranges for CSF WBC and CSF protein for each week category.

For CSF glucose, the examination of the 2002 cohort produced a Chi-Square statistic and p-values of 10.79 and 0.2138, respectively. For the 2008 cohort, the Kruskal-Wallis test produced a Chi-Square statistic of 7.541 and a p-value of 0.4795. These values indicate that no significant differences exist between glucose for the age stratifications in either cohort. In an attempt to see whether significant differences existed among broader age categories, the same tests were repeated on the data stratified by age in months. The results of these tests were also found to be not significant. This indicates that for CSF glucose no significant differences exist

between birth and 8 weeks of age, and so a single set of values should be calculated for each cohort.

3.4 GENERATING MEANS AND CONFIDENCE INTERVALS FOR CSF WBC, CSF GLUCOSE, AND CSF PROTEIN

One of the main goals of this research study was to generate a single set of clinically relevant means and confidence intervals that would guide physicians in knowing what normal values are for infants less than two months of age. Because CSF WBC, glucose, and protein were not normally distributed, methods based on normality could not be used to generate 95% confidence intervals. In order to obtain these values, bootstrap analysis was used. Bootstrap analysis is a non-parametric method that does not depend on a normal distribution to generate confidence intervals of the means (for more discussion, see materials and methods). The means were calculated using standard descriptive analysis. The means from standard descriptive analysis and 95% confidence intervals from the bootstrap analysis for CSF WBC and protein can be seen in Tables 21 and 22, respectively.

Table 21: Means and 95% Confidence Intervals for CSF WBC for all Cohorts Combined.

Age (weeks)	Mean WBC (cells/mm^3)	Lower Confidence Interval	Upper Confidence Interval
0-1	8.63	6.73	10.83
1	3.87	3.52	5.37
2	4.89	3.70	6.09
3	4.54	3.31	5.22
4	4.33	2.35	4.38
5	3.02	2.22	5.59
6	3.17	2.29	4.12
7	3.15	2.16	3.93
8	2.22	1.83	4.78

Table 22: Means and 95% Confidence Intervals for CSF Protein for all Cohorts Combined.

Age in Weeks	Mean Protein (mg/100mL)	Lower Confidence Interval	Upper Confidence Interval
0-1	106.44	94.12	115.22
1	77.61	75.45	88.19
2	71.03	64.74	77.85
3	68.68	63.31	79.42
4	62.15	57.77	66.70
5	60.15	55.33	65.11
6	53.35	49.25	57.73
7	63.63	55.46	73.38
8	54.18	44.97	60.97

The same methods were used to generate values for CSF glucose, although only mean and set of confidence intervals was generated for each cohort. The means and 95% confidence intervals for the 2002 and 2008 cohorts for CSF glucose can be seen in Table 23.

Table 23: Means and 95% Confidence Intervals for CSF Glucose for the 2002 and 2008 Cohorts.

Cohort	Mean Glucose (mg/100mL)	Lower Confidence Interval	Upper Confidence Interval
2002	52.97	51.72	54.37
2008	46.99	45.71	48.33

3.5 GENERATING NORMAL RANGES FOR CSF WBC, CSF GLUCUSE, AND CSF PROTEIN

The final question that we wanted to address in this study was "What values could physicians expect to see for normal infants?". That is, "How high is too high?" in infants less than two months of age. In order to answer this question, we set out to create a set of values for each variable that could be used by physicians to help better diagnose and initiate treatment in infants presenting with fever in emergency rooms. For CSF WBC and protein, these ranges were compiled among the combined cohort for each week stratification. For CSF glucose, ranges were calculated for the 2002 and 2008 cohorts. Because physicians are mainly concerned with the high-end ranges for CSF WBC, only the upper-percentiles will be reported for this variable.

CSF glucose and protein will include both upper and lower percentiles, as values can be elevated or depressed, depending on the condition. The values for these variables can be seen in Tables 24, 25, and 26 for CSF WBC (cells/mm^3), protein (mg/100mL), and glucose (mg/100mL), respectively.

Table 24: Medians, 75th, and 90th Percentiles for CSF WBC for all the Cohorts Combined.

Age (weeks)	CSF WBC (cells/mm^3) Median	75th percentile	90th percentile
0-1	6.0	9.0	26.0
1	3.0	6.0	9.0
2	3.0	7.0	9.0
3	2.0	4.0	9.0
4	2.0	4.0	8.0
5	2.0	3.0	6.0
6	2.0	3.5	8.0
7	2.0	3.0	7.0
8	2.0	3.0	7.0

Table 25: 10th, 25th, Median, 75th, and 90th Percentiles for CSF Protein for all Cohorts Combined.

	CSF Protein (mg/100mL)				
Age (weeks)	**10th percentile**	**25th percentile**	**Median**	**75th percentile**	**90th percentile**
0-1	62.0	78.0	98.0	132.0	153.0
1	53.0	64.0	76.0	86.0	103.0
2	44.0	56.0	66.0	84.0	106.0
3	43.0	53.0	68.0	78.0	85.0
4	38.0	49.0	59.0	74.0	84.0
5	37.0	43.0	56.0	75.0	85.0
6	32.0	41.0	48.0	64.5	84.0
7	34.0	43.0	50.0	76.0	105.0
8	29.0	38.5	50.5	62.0	84.0

Table 26: 10th, 25th, Median, 75th, and 90th Percentiles for CSF Glucose for the 2002 and 2008 Cohorts.

	CSF Glucose (mg/100mL)				
Cohort	**10th percentile**	**25th percentile**	**Median**	**75th percentile**	**90th percentile**
2002	43.0	47.0	51.0	56.0	62.0
2008	38.0	42.0	46.0	50.0	60.0

4.0 DISCUSSION

The generation of a set of standard values for CSF parameters in infants has long been an area of focus among health care professionals. This set of values can be useful in aiding in the early diagnosing and management of febrile infants who may have bacterial or viral meningitis. Although the risk of meningitis in the United States has dropped in recent years due to the widespread use of vaccines for some of the causative agents, including *H. influenzae* and Group B Streptococcus, the risk of meningitis to infants is substantial. This study utilized three independent chart reviews to produce sets of values for each of these cohorts as well as analyze if it was appropriate to pool the observations over time.

Using a sample size far larger than previous studies, this study proved that lab methods have remained constant through time for interpreting CSF WBC and CSF protein levels. This study also generated means, 95% confidence intervals, and expected percentiles for these surrogate markers of infection. CSF glucose values were found to vary between cohorts, but were not found to vary within cohorts. Because of this, values were generated for CSF glucose for the 2002 and 2008 cohorts separately. Because these values did not follow a normal distribution and generally could not be transformed to generate a set of values that did, non-parametric methods were employed to generate these confidence intervals and assess for differences among age in weeks for the surrogate markers of infection. CSF WBC, glucose, and protein are relatively easy measures for physicians to obtain and analyze in infants who present

with fevers in emergency rooms. It has long been known that these values vary over time in infants, although it was not certain to what degree and in what time frames differences should be assessed.

Elevated WBC counts in the CSF are typical indicators of infection, whether it be of bacterial or viral origin. In addition, CSF WBC counts appear to be elevated directly following birth. One of the major problems in the field of emergency pediatrics has been the uncertainty surrounding the questions, "How high is too high?" or "What is the normal expected value?" for these parameters in CSF. In our study, we found that CSF WBC counts are the highest directly following birth. This phenomenon has been explained in a number of ways, including the possibility that the blood-brain barrier is far more permeable directly following birth than later in life[10-14]. Another hypothesis to explain the elevated WBC count is that the actual process of being born may be "traumatic" to the infant. The stress of being born can lead to inflammation of the infants' meninges and the innate immune system releasing more cells to the CSF, even though no infection is present to warrant their presence. As the infant ages, the WBC values decrease as the blood brain barrier matures and becomes less permeable, and as the infant recovers from the birthing process. Previous studies, such as those conducted by Samson, Naidoo, Sarff, Pappu, Portnoy and Olson, Spanos, and Bonadio neglected to consider infants stratified by weeks of age[4,5,11,13,24,25]. Their methods of analysis included only examining infants one day old, less than one week, by month, or by three months.

This study shows that significant differences exist for infants in the first 8 weeks of life, and that important differences exist between infants who are 1 week old as compared to those who are 4 and those who are 8 weeks of age. We found significant differences between age in weeks for combined cohorts in terms of CSF WBC and protein. These tests formally examined

the means of the variables. This indicates that infants less than two months of age should not be compared to each other using a standard set of values for CSF WBC and protein. The means and 95% confidence intervals calculated for CSF WBC values in the combined cohort can be seen in Table 21.

From this table, we are able to see the value in stratifying infants by age in weeks as compared to making broader classifications of infants in terms or age. From birth to 2 weeks of age, we see a difference between the means of nearly 4 cells/mm^3 in the CSF WBC, with potential differences of 5 cells/mm^3 existing when comparing confidence intervals. From birth to 8 weeks of age, we see a difference between the means of over 6 cells/mm^3. These differences are clinically significant and it is therefore important to not classify these infants into broad age groups.

Additionally, it may be important for physicians to understand what the typical range of values will be for infants. For CSF WBC, the lowest that is possible is 0 cells/mm^3, which may not be unexpected. The upper ranges, however, can be quite high and also vary considerably between the week stratifications. Because physicians are majorly concerned with elevated CSF WBC values, the medians, 75^{th}, and 90^{th} percentile values for CSF WBC were calculated. The results of these calculations can be seen again in Table 24.

Like the means and confidence intervals calculated for CSF WBC, we see that the median, 75^{th}, and 90^{th} percentiles drop as age increases. The rates of decrease are not as obvious for the medians and 75^{th} percentiles, but a large drop is seen in the 90^{th} percentile between infants 0-1 weeks of age and infants 1 week of age. This again reinforces the importance of looking at infants stratified by weeks in age when attempting to diagnose or decide on early treatment options.

CSF protein values were also combined across cohorts and used for the generation of a standard set of values. The mechanisms for CSF protein transfer are similar to those for WBC in infancy. Although it is unknown why CSF protein levels are higher directly following birth, it has been postulated that the difference can be partially attributed to the immaturity of the blood brain barrier[10-14]. This temporal trend in protein levels was seen by Widell in 1958. His study found that protein levels were dramatically higher in the first two months of life. He did not stratify infants by week of age and generate means and confidence intervals, however. Extending upon his work, we found that the trend seen in CSF WBC largely parallels that for CSF protein. Large differences exist when comparing infants who vary in age by only a few weeks. The means and 95% confidence intervals generated for the infants in this study can be seen in Table 22.

The differences between protein levels in infants of varying age are even more pronounced than the trends that were seen for CSF WBC. When comparing infants ages 0-1 week and 1 week of age, there is a mean difference in protein of 25 mg/100mL with the potential difference being as large as 40 mg/100mL. This trend increases as age increases, with the mean difference between infants 0-1 weeks of age and 8 weeks of age being approximately 50 mg/100mL and the largest potential difference being 55mg/100mL. These differences are clinically significant and would potentially be missed if infants were grouped according to broader age criteria.

Knowing a reference range of normal values for CSF protein is also important for physicians. Unlike CSF WBC, there could be situations where CSF protein levels could drop. It was decided for this reason to not only include upper tail values for CSF protein, but also include

lower tail values. The 10th, 25th, median, 75th, and 90th percentiles for CSF protein for the combined cohort can be seen in Table 25.

As seen by the expected range of values, large differences also exist between newborn infants and those a few weeks old. Specifically, a large decrease is seen between all percentiles between infants ages 0-1 week and 1 week of age. This trend is seen throughout the cohort as age in weeks increases, although the differences begin to level out as the 8 weeks is reached. Similarly for CSF WBC, this again reinforces the idea that infants under 2 months of age should not be evaluated using the same set of diagnostic values for all age groups.

The values for CSF glucose did not follow the same temporal trends as CSF WBC and CSF protein. When analyses were being run to determine whether it was appropriate to pool the observations across cohort, there were some differences among CSF glucose values depending on the age in weeks between 2002 and 2008. For ages 3 weeks, 5 weeks, and 6 weeks, significant differences existed between the values obtained for the 2002 and 2008 cohorts. Nearly significant differences also existed for other weeks, although due to the Bonferroni adjustment they did not make the cutoff. In order to determine a clinically relevant reason for why this difference existed, the University of Pittsburgh Medical Center's Laboratory was consulted to see if they had changed their standards of analysis. They reported that although they had not changed their methods, they too had noticed a changing trend among CSF glucose values over time. They indicated that they had noticed that over years the CSF glucose values had been decreasing. This trend was also seen in our data analysis. CSF glucose values for the 2002 cohort were higher than those for the 2008 cohort. It follows that some outside source is likely causing a variation in population level CSF glucose values that is not related to collection or lab analysis. Because the reason for these differences could not be identified, it was decided

to not pool the observations, even though the overall trend was to pool the observations. Therefore, CSF glucose was analyzed individually for the 2002 and the 2008 cohorts.

When non-parametric ANOVA was run on the 2002 and 2008 cohorts for CSF glucose, no statistically significant differences existed within cohorts for the values when stratified by age in weeks or age in months. It has been discussed in the literature that CSF glucose levels are different in infants as compared to adults, although our data suggest that from birth to two months there are relatively few changes in the range of CSF glucose. For these reasons, CSF glucose means and confidence intervals were generated for the entire cohorts, independent of age in weeks or months. The means and 95% confidence intervals for CSF glucose among the 2002 and 2008 can be seen in Table 23.

Looking at these values, we see the trend that was identified by the University of Pittsburgh Medical Center's Laboratory. Values for the 2008 cohort are lower than those for the 2002 cohort. Because the observations were not able to be pooled, we were unable to generate a single mean and set of confidence intervals that would be appropriate to use when diagnosing infants.

Similarly to CSF protein, CSF glucose values could also be expected to be higher or lower than normal depending on the cause of infection. As discussed in the introduction, CSF glucose values can vary even depending on the causative agent for meningitis. In viral meningitis, CSF glucose values tend to be increased, but in bacterial meningitis, they are decreased. It was therefore important to generate a wide range of expected values for CSF glucose. The 10^{th}, 25^{th}, median, 75^{th}, and 90^{th} percentiles for CSF glucose can be seen in Table 26.

Looking at these values, it does not seem as though there are large differences between the two cohorts in terms of expected ranges, although overall the same trend is upheld: CSF glucose values are higher in the earlier cohort. Again, because we were not able to pool observations between the two cohorts so we were not able to generate a single set of values for the range.

More research will need to be undertaken to understand the changes differences CSF glucose values in the different cohorts. Because lab practices have not changed in the manner in which CSF glucose is analyzed, it implies that there is an environmental reason why CSF glucose is decreasing in the population. In Pittsburgh, this trend can be examined by also examining serum glucose levels to see if they too have also been decreasing through the years. It is possible that changes in infants diet (such as changes to formulas, vitamins, etc) or rates of breastfeeding may affect both serum and CSF glucose ratios. It will be important to investigate whether this trend has been seen in other cities besides Pittsburgh. If this trend has not been recognized by other researchers, it is likely that there is something unique about our study population which is responsible for the differences. If data is available, cross sectional studies on existing data and new ones could be performed to determine if there is a relationship between the factors discussed (breast feeding rates and formula usage) and decreased glucose values.

Comparing our findings to previous studies that look at similar age strata as ours, we find that our estimates vary considerably. Widell's study looked at 48 infants and divided infants by age in weeks for the first two weeks and then divided them in two week segments following this[11]. For WBC, he found an average value of 7.5 cells/mm^3 for all infants, ranging from birth to 60 days[11]. He does not specify whether he chose to do this because no significant differences existed or for another reason. Widell did not look at CSF glucose, but he did examine total

protein levels. Total protein levels for Widell's age strata were significantly lower than ours. He found values of protein ranging from 80.9 to 34.8mg/100mL for infants ages 0-6 days and 42-59 days, respectively. Our range of values was 106.44 to 54.18 mg/100mL of protein for infants 0-1 weeks and 8 weeks, respectively. These differences could exist because Widell used a different method for analyzing protein or because different inclusion criteria were used.

The next study which has comparable age groupings is Bonadio *et al.*'s study from 1992. Their study examined 35 infants 0-4 weeks of age and 40 infants ages 4-8 weeks of age. Their WBC values were higher than ours, with a mean of 11.0 cells/mm^3 for infants ages 0-4 weeks and 7.1 cells/mm^3 for infants ages 4-8 weeks[21]. If we were to average our values together over 0-4 weeks and 4-8 weeks, we would find approximately 5 cells/mm^3 and3 cells/mm^3, respectively. Bonadio *et al.* also examined CSF glucose and CSF protein and it appears that our measurements would be comparable if we were using the same age stratification. Bonadio *et al.* completed this study in 1992, before the widespread use of PCR as a means of identification of viral pathogens that cause meningitis. It is possible that Bonadio *et al.*'s sample was subject to misclassification bias because some of his "normal" cases were actually undiagnosed cases of viral meningitis.

The final study that we can directly compare our findings to is Ahmed *et al.*'s study from 1996. Ahmed *et al.* found that for infants 0-7 days old, the mean CSF WBC value was 15.3 cell/mm^3 [14]. For their other age stratifications, 2 weeks, 3 weeks, and 4 weeks, his findings were higher than ours. This trend did not hold through for all the surrogate markers, however. The protein values we found were consistently higher for each age group. For glucose, the values found by Ahmed *et al.* matched ours relatively well. They found means for glucose that ranged from 45.9mg/100mL to 54.3mg/100mL[14]. Our overall finding was that for infants ages 0-60

days the mean value should be 50.09mg/100mL. It is difficult to say why differences exist in our study findings but Ahmed *et al.*'s study had far fewer infants. It is possible that different inclusion criteria were used that were not as stringent as ours. It is also possible that different techniques were used to measure CSF WBC and CSF protein, although this is not as likely as these methods are largely standardized.

A comparison of the findings from Widell's, Bonadio *et al.*'s, Ahmed *et al.*'s, and this study can be seen in Table 27.

Table 27: Summary of Results for Widell, Bonadio, Ahmed, and Chadwick.

Author	Year	Population	Age Groupings/ Strata	CSF WBC (cells/mm^3)	CSF Glucose (mg/100mL)	CSF Protein (mg/100mL)
Widell[11]	1958	48 children <2 months	0-6 days 7-13 days 14-27 days 28-41 days 42-59 days	7.5 (avg for all groups)	No Data	80.9 70.4 53.9 46.5 34.8
Bonadio *et al.*[13]	1992	75 infants < 8 weeks	0-4 weeks 4-8 weeks	11.0 7.1	46.0 46.0	84 59
Ahmed *et al.*[14]	1996	108 infants < 30 days	1 week 2 week 3 week 4 week	15.3 5.4 7.7 4.8	45.9 54.3 46.8 54.1	80.8 69.0 59.8 54.1
Chadwick, Martin, Wilson	2009	1,019 infants < 60 days for CSF WBC 530 infants <60 days for CSF glucose and CSF protein	0-1 Weeks 1 Week 2 Weeks 3 Weeks 4 Weeks 5 Weeks 6 Weeks 7 Weeks 8 Weeks	8.63 3.87 4.89 4.54 4.33 3.02 3.17 3.15 2.22	50.09	106.44 81.67 71.03 77.17 62.15 60.15 53.35 63.63 54.18

Although we feel that our overall study design was sound, there exist some limitations in our methods. A retrospective cohort is cost effective and efficient in terms of collecting data, as all the variables of interest exist already, but it does leave potential for information bias. By using such strict criteria for inclusion into the study, however, we attempted to limit the amount of information bias. Despite the application of these criteria, however, it is still possible that

some of the infants analyzed were misclassified. Specifically, in the 1995 cohort, PCR and other viral culturing methods were not as precise and developed as they are today. But we feel that limiting the inclusion of infants with less than 100 WBC/mm^3 limits the majority of potential viral meningitis cases. Additionally, we assume that we have found all of the cases available during the time periods selected for analysis. However, there still could be patients that we have missed. Our findings are designed in a way to attempt to apply them to all infant populations less than 2 months of age. However, infants in other parts of the country may differ from those in Pittsburgh.

Another problem that exists in our study is the limited number of infants available for analysis in the 8-week age group. In our entire study of 1,019 infants, only 36 were 8 weeks of age. Because our sampling was done using only infants less than 60 days of age, our 8 week year old category was not complete. This age range should have extended form 56-63 days, and thus infants ages 61-63 days were not included based on our less than 2 months of age criterion. This can explain why there is a discrepancy in the number of observations for this week category as compared to the others. Additional reasons why this age in week category could be lower than the other week categories is that possible that at this age, parents are less likely to bring their infants to the emergency room for fever because they are more comfortable with the presence of a fever and are no longer concerned with the risks of meningitis. Another possible reason is that infants' immune systems have matured enough at this point to prevent infection that could lead to meningitis. It is also possible that another reason exists which we have not considered. To examine these latter two explanations, it could be possible to see if the rates of infants ages 8 weeks of age is different for all visits to the emergency room, not just those that result in a lumbar puncture. Also, parents of infants could be surveyed to see if their opinions on

whether or not they would be likely to bring their child in for examination following the onset of a fever for various age categories. In the future, however, it might be possible to extend more infants 8 weeks of age by extending the cut-off from 60 to 63 days of age. This would probably compensate for the variation seen in this study and make the age stratifications more uniform.

Further research can now be done to determine how effective these values are at the early identification of meningitis. It is possible that another retrospective chart review could be beneficial to accomplish this goal. By identifying cases of meningitis retrospectively, the CSF WBC, glucose, and protein values can be compared to those that were found upon initial CSF fluid examination. By knowing how many infants with meningitis fall within our confidence intervals, we can get an idea of how accurate or inaccurate our values are for the application to "normal" infants. If this retrospective study finds positive results indicating that these values are accurate predictors of bacterial and viral meningitis, it is possible to then introduce these values into practice to determine if they benefit physicians attempting to diagnose meningitis quickly and easily. The use of a prospective study would be valuable in analyzing these objectives.

Regardless of the predictability of these values, however, it should be remembered that the CSF WBC, CSF glucose, and CSF protein are surrogate markers that can help identify the possibility of viral or bacterial meningitis, but they should not be used as a replacement for true diagnostic tests such as bacterial or viral culture or PCR. These values are designed to aid in the decision making process of whether to start early treatment for bacterial or viral meningitis.

In conclusion, we have performed the largest retrospective cohort to date of normal infants to generate values for CSF components that may be useful in clinical practice for early identification of infants who would benefit from early treatment before diagnostic tests can be completed. This study found that significant differences exist for CSF WBC and CSF protein for

infants of varying ages up to two months, but found no differences for CSF glucose. This study proves that infants of varying ages should be stratified by week in age when determining what is "normal" for CSF components.

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