Ability of Brain Natriuretic Peptide Tests and Homocysteine to Exclude Congestive Heart Failure

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Abstract: Among the most promising tests for early detection of congestive heart failure (CHF) are brain natriuretic peptides (BNPs), but it is unclear if homocysteine can aid in differentiating those with and without disease. We attempt to use a combination of tests to reduce the need for echocardiography without missing any cases of CHF.

We compared 50 CHF patients with an ejection fraction of less than 60%, to 100 patients with non-specific symptoms but with a normal echocardiogram. After setting sensitivity at 100%, specificities for BNP, amino-terminal probrain natriuretic peptide (NT-pro BNP), and homocysteine were 34%, 28% and 36% respectively. Combinations of two tests if homocysteine was included increased the specificity to 56%. The AUC for BNP and NT-proBNP were not significantly different (92.8 and 95.9, respectively) whereas the AUC for BNP and NT-proBNP tests were superior to homocysteine (83.2). Compared with performing echocardiography in all patients, savings were dependent on the pretest probability. At a 1% pretest probability of CHF, savings were 44.5% while using a combination of NT-proBNP with homocysteine test. Homocysteine can be used in combination with either of the BNPs to decrease costs of early detection of CHF.

Keywords: Homocysteine, brain natriuretic peptide, NT-proBNP, congestive heart failure, costs, comparison.

INTRODUCTION

Congestive heart failure (CHF) is a major health problem with inherent morbidity and increased mortality rates. If early detection and treatment of asymptomatic patients with CHF decrease the rate of progression to symptomatic disease [1], tests to rule out disease could be appropriate [2]. Echocardiography is the standard method for detecting CHF, but resources might not allow its widespread use since it is expensive and its performance and interpretation require expertise [3]. Furthermore there may be cost savings if relatively inexpensive blood tests were available with the ability to reliably rule out CHF.

Among the variety of tests, the most promising blood tests are the brain natriuretic peptides: brain natriuretic peptide (BNP) [1,4-9], and amino-terminal probrain natriuretic peptide (NT- proBNP) [8,10,11]. Plasma homocysteine might also contribute to ruling out CHF, since homocysteine has been shown to be a risk factor for developing CHF, and plasma levels are associated with the severity of the disease [12,13]. We are unaware however of studies comparing the ability of homocysteine with that of brain natriuretic peptides to identify patients with CHF, without missing any cases of disease.

In the following study we assess and compare the ability of these three blood tests alone and in combination to reduce the need for echocardiography without missing any cases of CHF.

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METHODS

Patients

The study was approved by the Ethics Committee of Lady Davis Carmel Medical Center, and Informed consent was obtained from each patient. We compared two groups of patients referred for echocardiography. The study group included 50 patients with a history of treatment for CHF and an EF of less than 60% at the time of testing. Forty of the 50 patients had an EF at time of blood testing of less than 40%, where the others were between 45-55%. They were compared to 100 consecutive patients with nonspecific symptoms, such as shortness of breath either at rest or on exercise, chronic dry cough, leg swelling, or severe fatigue but with an EF of 60% or more [14], and no history of treatment for CHF.

Materials

Blood samples were drawn by vacutainer EDTA plastic tubes (Greiner bio-one, Kremsmuenster, Austria), and centrifuged within 4 hours. Plasma samples were aspirated and transferred into plastic tubes that were stored at -70^0C until analysis.

BNP was assayed by Abbott BNP immunoassay that was preformed on Axsym analyser according to manufacturer's recommendations; Abbott (Wiebaden-Delekenheim, Germany). NT-proBNP was performed on Immulite 2000 ac-

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cording to manufacturers instructions; DPC (Los Angele, CA).

Homocysteine was detected by Abbott Homocysteine Fluorescence Polarization Immunoassay that was preformed on Axsym analyser according to manufacturer's recommendations; Abbott (Wiebaden-Delekenheim, Germany).

Statistical Analysis

We used MedCalc (Version 11.1.1, MedCalc Software, Mariakerke, Belgium) for statistical analysis. Mean values of the various tests in the two groups were compared with a one sided t-test, a more sensitive method to detect significant differences in the expected direction. A p value of less than 0.05 was considered significant. Next, the cut off value for each test was determined by fixing the sensitivity at 100%. Given 100% sensitivity, we considered combinations of parallel tests to determine if specificity might be increased. Then we used the given specificities to calculate the possible cost savings that might be obtained without missing any patients with congestive heart failure. We assumed that an echocardiography costs 420 US$ [15], BNP test 32 US$ [15], NT pro-BNP test 20 US$ [9], and homocysteine test 22 US$ [16] and calculated costs at different pretest probabilities and savings comparing the use of blood tests with selective use of echocardiography to the use of echocardiography on all patients. We also compared the areas under the receiver operator curve (AUC) for the three tests in order to compare the overall ability of the tests to differentiate between those with and without CHF at all possible cut-off values.

RESULTS

Age was similar in the two groups, whereas for all the tests, mean values were significantly higher in the patients with CHF (Table **1**). After setting the cut-off value to give a 100% sensitivity, the specificities for BNP, NT-pro BNP, and homocysteine were 34%, 28% and 36% respectivity (Table **2**). Combinations of two tests if homocysteine was included increased the specificity to 56%, and with three tests 63%. The AUC for BNP and NT-proBNP were not significantly different (92.8, 95% confidence interval (CI) =87.4-96.4, and 95.9, 95% CI 91.3-98.4 respectively) whereas the AUC for BNP and NT-proBNP tests were superior to homocysteine, 83.2% (95% CI 76.2-88.8, p=0.026 and p=0.002 respectively). If we used a cutoff of EF<45% to define CHF, then the specificity of the NT-proBNP assay was increased from 28% to 84%, and the BNP assay from 34% to 83%, whereas the specificity for homocysteine was not improved.

Savings were dependent on the pretest probability, with a savings of 44.5% using a combination of NT-proBNP and homocysteine tests at 1% pretest probability which decreased to 18% if the pretest probability was 50% (Table **3**). If the cost of echocardiography decreased to around 120$, there would be no savings (calculation not shown).

DISCUSSION

We found that the specificity of homocysteine is comparable to the brain natriuretic peptides in identifying those without CHF after requiring 100% sensitivity. Our results are consistent with previous studies showing that homocysteine is a risk factor for CHF as well as being associated with

Table 1. Comparison of the Various Tests in those with and without Congestive Heart Failure

Variables	Units	No CHF N=100	CHF N=50	P value
Age	years	67.9±1.1*	68.7±1.6	0.6913
Homocysteine	µmol/L	11.5±0.5	18.5±0.7	<0.001
BNP	ng/L	86±48	744±68	<0.001
NT pro BNP	ng/L	155±261	3424.5±370	<0.001

*Mean ± standard error.

Table 2. Comparing the Specificities in the Best Differentiating Tests Using a Cutoff of 100% Sensitivity

Test	Specificity (%) N=100	Specificity (%) 95% CI	Cut-off Value
BNP	34	24.8-44.2	24.8 ng/L
NT-pro BNP	28	19.5-37.9	34.4 ng/L
Homocysteine	36	26.6-46.2	9.61 µmol/L
NT-proBNP + BNP*	44	34.1-54.3	
BNP+ homocysteine	56	45.7-65.9	
NT-proBNP + homocysteine	56	45.7-65.9	
BNP+NT-proBNP+ homocysteine	63	52.8-72.4	

*Compared to other two combinations individually (p = 0.08).

Table 3. Cost Analysis for Diagnosing and Ruling Out Congestive Heart Failure with Various Combinations of Blood Tests and Echocardiograms (US Dollars)

Pretest	Tests	Costs Times Number	Echocardiogram Total Number	Costs of Echo[†]	Total Costs	Savings %
	0	**0**	**200**	**84000**	**84000**	**0**
1%	1*	22x200=4400	127+2=129[‡]	54180	58580	31.3%
	2	42x200=8400	87+2=89	37380	45780	44.5%
	3	74**x200=14800	73+2=75	31500	46300	43.9%
5%	1	22x200=4400	122+10=132	55440	59840	28.8%
	2	42x200=8400	84+10=94	39480	47880	43.1%
	3	74x200=14800	70+10=80	33600	48400	42.4%
20%	1	22x200=4400	102+40=142	59640	64040	23.8%
	2	42x200=8400	70+40=110	46200	54600	35.5%
	3	74x200=14800	59+40=99	41580	56380	32.9%
50%	1	22x200=4400	63+100=163	68460	72860	13.3%
	2	42x200=8400	44+100=144	60480	68880	18.0%
	3	74x200=14800	37+100=137	57540	72340	13.9%

*Assuming specificities of 36% for one test (homocysteine), 56% for two tests (one of the brain natriuretic peptides and homocysteine), and 63% for three tests.
[†]Cost of blood tests in order of addition is 22$ for homocysteine, 20$ for NT- proBNP, and 32$ for BNP = together 74$. cost of echocardiogram = $420.
[‡]Number of echocardiograms is calculated by assuming that all patients with disease are positive by screening and therefore the number = the prevalence, whereas the number of tests for those without disease is dependent on the one minus the specificity (e.g. 200 X 1% = 2, and 1-0.36 times 198 (those without disease) = 127); thus the total number of echocardiographs here = 127 + 2 or 129.

the severity of the disease [12,13,17]. However it is not unexpected that the AUC for homocysteine although high (83%), was significantly less than that of the Brain natriuretic peptides, since levels are not specific and have been shown to correlate with serum folate, B12, and creatinine levels [18]. The cutoff level of 9.6 μmole/L for homocysteine is slightly lower than that suggested as the lower relative value for those with good vitamin status (12 μmole/L) [12].

Our study is not directly comparable to most other studies that used EF cutoff values of 30-50% [4] to define CHF. We used a cutoff of less than 60% in order to insure that patients with mild congestive heart failure were not included in the control group. This would be likely to decrease specificity of the tests. In fact if we used a cutoff of EF<45% to define CHF, then the specificity of our NT-proBNP assay increased from 28% to 84%, the BNP from 34% to 83%. At 100% sensitivity other studies found variable BNP specificities of around 30% [7], close to zero [6], 40%[8] and 19.6%[15], whereas for NT-proBNP a specificity of around 70% was found in one study [8] and 61% in another [19]. The variable results are probably due to patient selection and might also be due to differences in the various BNP assays [20]. Nevertheless our cutoff value for BNP of 24.8 ng/L is similar to that of other studies using different BNP assays and definitions of normal cardiac function [7,19], but would have increased to 71 ng/L if CHF was defined as an EF of less than 45%. For NT-proBNP a cutoff value of 358 ng/L was reported in one study [19] whereas we found a very low value of 34.4 that increased to 185 if abnormal cardiac function was defined as less than 45%. Further studies with large numbers of patients are clearly needed to more precisely define appropriate cutoff values.

We also found that a combination of NT-proBNP with homocysteine can lead to 44.5% savings compared to doing echocardiography in all patients with a pretest prevalence of 1%, but savings decrease with increased pretest prevalence. There would be no savings if echocardiography with a cost of 120$ per test was widely available.

Our results should be interpreted with caution. Methodological differences might lead to different results. Furthermore our selection of patients with CHF might represent a spectrum bias with 80% having an EF of less than 40%. Specificity might be lower if more patients with EFs of between 40-60% are included. Still our results may be relevant to clinical practice since an EF of less than 40% is often used to determine when it is appropriate to treat asymptomatic patients in order to prevent progression to symptomatic CHF [1]. Finally the Israeli population eats a Mediterranean diet rich in vitamins and extrapolation to other populations with different diets might not be warranted with regards to homocysteine.

There is no consensus on recommending screening of asymptomatic populations or case finding in those with non-specific symptoms. Randomized controlled trials are needed to determine if there is decreased morbidity and/or mortality in those found with CHF treated earlier than in the control group. Our study suggests that plasma homocysteine should

be included in the screening/case finding tests for such a study.

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