results. Performing a LightCycler assay in a closed system eliminates post-amplification processing. This considerable reduction in the number of manipulations reduces the risk of contamination. The use of the LightCycler clearly reduced analysis time (45 minutes vs. 1.5 days).
In conclusion real-time PCR followed by melting curve analysis is a rapid, simple, accurate method for genotyping the VKORC1 1173C>T polymorphism.

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Automated result interpretation in anemia testing using artificial neural networks

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Artificial neural networks (ANNs) are non-linear statistical data processing tools based on the simulation of groups of interconnected neurons, which work analogously to biological neural nets. An ANN can be trained for a particular task by repetitively adjusting inter-neuronal connection weights so that the discrepancies between output and true values are minimized. ANNs can be applied in complicated classification tasks, and show promise for application in medical decision making (1,2). In our laboratory, anemia test results ordered by general practitioners are reported with classification codes referring to the most probable cause of anemia. The coding is done non-automated by a clinical chemist, is time intensive and probably operator dependent. In this study, we examined the abilities of two ANNs software packages to learn this particular task, in order to explore the feasibility of automated interpretation of laboratory results.

Methods

Two software programs were used, both being implementations of a standard feed-forward back-propagation ANN model. Nets were created with one input layer containing a number of neurons equaling the number of input parameters, two hidden layers with variable numbers of neurons, and one output neuron signaling the likelihood of a particular classification. Dedicated ANNs were employed in parallel for the classification codes: iron deficiency, thalassemia, infection, blood loss, pregnancy, decreased erythropoiesis, and uncertain/unknown cause. The general principle is shown in figure 1. Separate datasets were used for training, for validation (detecting potential overtraining) and for evaluation, and only contained test results ordered by general practitioners. Data were obtained from the LIS and a Sysmex XE-2100 hematology analyser.
The first program tested was NNclass, a freeware MS-Excel implementation (3). Nets were defined with randomly sized hidden layers. Input parameters included: age, sex, ESR, zinc protoporphyrin (ZPP), Hb, RBC, MCV, MCH, RDW-SD, neutrophilic granulocytes, immature reticulocytes fraction and reticulocytes. Missing input data were substituted by mean values obtained from the training dataset. Training was performed for 2000 cycles using 649 training and 115 validating examples. ANNs with the lowest training- and validation error rates were selected, feeded with an evaluation dataset (n=170), and evaluated for the subjective acceptability of their output. The analytical performance was evaluated with a separate dataset (n=431).
EasyNN-plus (4), a commercial stand-alone application, was also tested. Hidden layer size was optimized by the program prior to learning. Input parameters were: age, sex, ESR, ZPP, Hb, RBC, MCV, RDW-SD, platelets, granulocytes, lymphocytes, monocytes, IRF and reticulocytes. Missing input data were substituted by median values

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Table 1. Analytical evaluation of trained ANNs. Freq: frequency of that classification category in the evaluation set. Sens: sensitivity, Spec: specificity, PPV: positive predictive value, NPV: negative predictive value.

		NNclass Training : n=649 Evaluation : n=431				**EasyNN-plus** Training : n=1274 Evaluation : n=431			
	Freq	**Sens**	**Spec**	**PPV**	**NPV**	**Sens**	**Spec**	**PPV**	**NPV**
Iron deficiency	28.3%	0.84	0.90	0.78	0.93	0.74	0.95	0.86	0.90
Thalassemia	10.4%	0.58	0.96	0.63	0.95	0.56	0.98	0.81	0.95
Infection	5.8%	0.48	0.95	0.36	0.97	0.60	0.95	0.44	0.97
Blood loss	6.5%	0.64	0.97	0.58	0.98	0.61	0.96	0.49	0.97
Pregnancy	5.1%	0.50	0.99	0.75	0.97	0.68	0.98	0.63	0.98
Decreased erythropoiesis	1.9%	0.75	0.98	0.35	1.00	0.75	0.97	0.35	1.00
Uncertain/unknown	64.5%	0.90	0.66	0.84	0.78	0.90	0.68	0.84	0.79

obtained from the training dataset. Nets were trained using 1274 training and 223 validating examples until validation error increased (typically between 1000-10,000 cycles). ANNs with the best sensitivity en specificity were evaluated (n=431) for analytical performance.

Results

Multiple (10-80) ANNs were trained per anemia classification code, and the best of each type were used for analytical evaluation. Results are summarized in Table 1. Selected ANNs generally had moderate sensitivity (ranging 0.48-0.90) and good specificity (>0.95). Although positive predictive values were moderate (range 0.35-0.86), negative predictive values were good (range 0.78-1.00). In overall, both ANNs programs gave comparable results. In addition to the analytical evaluation, the ANNs created with NNclass were also evaluated for the acceptability of their errors. Out of 170 anemia classifications generated by the selected ANNs, 36 (21%) deviated in one or more ways from the human-made classification. Of these deviations, only 6 (3.5% of total) were considered to be critical errors as subjectively judged by an experienced clinical chemist.

Conclusions

An obvious approach for automation of classification tasks would seem to be the use of flowcharts.

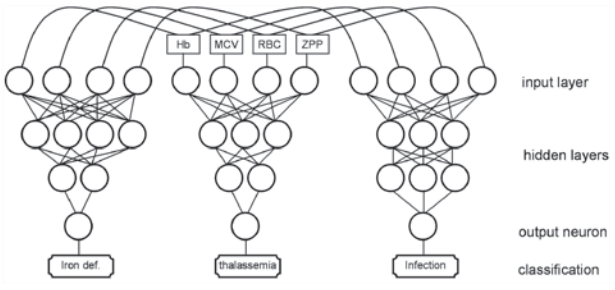

Figure 1. Simplified representation of dedicated ANNs for each anemia class being used in parallel.

Although flowcharts do have advantages such as transparency, it is difficult to document the complex and fuzzy manner of human decision-making. Moreover, results of flowcharts used in anemia classification are often sub optimal (5). We therefore tried ANNs as alternative approach. When employing ANNs, it is essential to prevent overtraining which will result in poor generalization power (6). By cross-validation against a separate evaluation dataset we demonstrate that selected ANNs could also classify new cases that were not used in the training process. Although both NNclass and EasyNN-plus produced ANNs with equal analytical performance, the author prefers EasyNN because of its operating speed. Resulting ANNs combine moderate sensitivity with high specificity, implicating that they are rather conservative in detecting anemia causes. This may well be a desirable property when providing result interpretation to general practitioners. The amount and type of errors produced by the ANNs are acceptable. We conclude that the use of ANNs in anemia classification in a laboratory setting is feasible. Some issues, however, need further investigation, for example long term stability of analytical performance and the question of how to implement ANNs in daily laboratory routine.

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