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Prediction of drug absorption based on immobilized artificial membrane (IAM) chromatography separation and calculated molecular descriptors

T.E. Yen [a], S. Agatonovic-Kustrin [b,*], A.M. Evans [a], R.L. Nation [c], J. Ryand [d]

[a] *Centre for Pharmaceutical Research, University of South Australia, Adelaide, SA, Australia*
[b] *School of Biomedical and Chemical Sciences, Pharmacy, UWA, Crawley, WA, Australia*
[c] *Victorian College of Pharmacy, Monash University, Parkville, Vic., Australia*
[d] *Biota Holdings Limited, Melbourne, Vic., Australia*

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Abstract

The aim of this study was to e valuate the usefulness of IAM chromatography in building a model that would allow prediction of drug absorption in humans. The human intestinal absorption values (%HIA) for 52 drugs with low to high intestinal absorption were collected from the literature. The retention (capacity factor, k') of each drug was measured by reverse-phase HPLC using an IAM.PC.DD2 column (prepared with phosphatidylcholine analogs, 12 μM, 300 Å, 15 cm × 4.6 mm) with an eluent of acetonitrile–0.1 M phosphate buffer at pH 5.4. In addition, 76 molecular descriptors and solubility parameters for each drug were calculated using ChemSW from the 3D-molecular structures. Stepwise regression was employed to develop a regression equation that would correlate %HIA with molecular descriptors and k'.

Human intestinal absorption was reciprocally correlated to the negative value of the capacity factor ($-1/k'$) ($R = 0.64$). The correlation was further improved with the addition of molecular descriptors representing molecular size and shape (molecular width, length and depth) solubility (solubility parameter, HLB, hydrophilic surface area) and polarity (dipole, polar surface area) ($R = 0.83$).

Experimentally measured IAM chromatography retention values and calculated molecular descriptors and solubility parameters can be used to predict intestinal absorption of drugs in humans. Developed QSAR can be used as a screening method in the designing of drugs with appropriate IA and for the selection of drug candidates in the early stage of drug discovery process.

Keywords: IAM chromatography; Drug absorption; Molecular descriptors; QSAR

1. Introduction

Successful drug development requires optimization of both the pharmacological activity of the drug at the target receptor site, and the delivery to that site. Oral delivery is the most popular and convenient way of drug administration, and the final plasma concentrations of an orally administered drug depend critically upon its gastro intestinal absorption. Thus, understanding which properties need to be optimized in order to enhance oral absorption has become the subject of early stage preclinical research in medicinal and/or combinatorial chemistry settings. Poor intestinal permeability of drugs constitutes a major bottleneck in the successful development of candidate drugs and a number of in vivo and in vitro methods has been developed in order to predict intestinal drug absorption. A drawback for most of these methods is that they are time consuming and therefore have a limited throughput. Several computational approaches have been published but there is a lack of a general strategy. Thus, there is still a need for development of fast methods for physicochemical screening, correlated to drug absorption and consequently to drug bioavailability.

For a drug to be absorbed from the gastro intestinal tract following oral administration, it must be capable of moving across cell membranes (transcellular absorption) or between

* Corresponding author. Present address: School of Biomedical and Chemical Sciences, M315 Pharmacy, The University of Western Australia, Crawley, WA 6009, Australia. Tel.: +61 8 64883317; fax: +61 8 64881025.
E-mail address: nkustrin@cyllene.uwa.edu.au (S. Agatonovic-Kustrin).

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tight gaps in between cells of the gastro intestinal mucosa (paracellular absorption) to the circulation on the other side. Both transcellular and paracellular diffusion depend upon the properties of drug molecules, such as molecular size, polarity, and lipophilicity [1]. Membranes of the gastro intestinal tract are biologic barriers that selectively inhibit the passage of drug molecules and are composed primarily of a bimolecular lipid matrix, containing mostly cholesterol and phospholipids. Absorption from the gastro intestinal tract, as well as penetration of other membrane barriers may be passive or active. Passive transport is governed by physicochemical properties whereas active transport involves specific binding of a molecule to a binding site on a transport protein.

Immobilized artificial membrane (IAM) chromatography has recently gained acceptance as a chromatographic method for the estimation of the membrane permeability of small molecule drugs [2]. IAMs are chromatographic surfaces prepared by covalently immobilizing cell membrane phospholipids to solid surfaces at monolayer densities [3]. Since phosphatidylcholine (PC) is the major phospholipid found in cell membranes, IAM surfaces prepared from PC analogs mimic the phospholipids environment found in the cell membrane. They simulate the hydrophobic and hydrophilic contribution of drug-membrane partitioning and can be used to as a fast screening column for predicting drug absorption [4]. On the other hand, studies of the relationships between the chemical structure of drugs (steric and electrostatic fields) and their affinity for the small intestinal oligopeptide carriers have shown that carrier mediated permeability is sensitive to composition, size and hydrophobicity of the ligands [5,6].

In this context, the aim of the present study was to develop a model that can predict gastrointestinal absorption for a diverse range of drugs using a combination of immobilized artificial membrane retention properties [7,8] and selected theoretical physicochemical drug descriptors [9].

2. Materials and methods

2.1. Test compounds

The drugs were purchased from Sigma (St. Louis, MO, USA). Water-soluble drugs were dissolved and diluted in 0.1 M phosphate buffer solution (PBS) (pH 5.4) to appropriate concentration and lipophilic drugs were first dissolved in methanol and then diluted with 0.1 M PBS to appropriate concentration.

2.2. Chromatographic system

The HPLC system comprised a SCL-10A system controller, a LC-10AD pump, a SIL-10A*vp* auto-injector (Shimadzu, Kyoto, Japan) and a SPD-10AV UV–vis spectrophotometric detector (Shimadzu, Kyoto, Japan) connected to a C-R6A integrator (Shimadzu, Kyoto, Japan).

2.3. Immobilized membrane chromatography

The retention (capacity factor, k') of 52 drugs with low to high intestinal absorption in humans was determined by reverse-phase HPLC using an IAM.PC.DD2 column (prepared with PC analogs, 12 mM, 300 Å, 15 cm × 4.6 mm) (*Regis Technology*, Morton Grove, IL, USA) with an eluent of acetonitrile–0.1 M phosphate buffer at pH 5.4 (0/100-45/55, v/v). The mobile phase was filtered (0.45 μm, Milford, MA, USA) and degassed in an ultrasonic bath (Soniclean Ltd., Stepney, South Australia) prior to use. All experiments in this study were carried out at a room temperature (23 ± 2 °C) using the flow rate of 1.0 ml/min. The ratio of acetonitrile–PBS and detection wavelength varied for individual drugs (UV wavelength was 210–278 nm). For drugs with low UV absorbance, such as mannitol, radiolabelled compounds were used for the experiments and a radiometric detector was used instead of a UV detector.

2.4. Capacity factor

In order to eliminate the impact of the dead volume of the chromatographic system, the capacity factor (k') was calculated by the following equation:

$$k' = \frac{t_r - t_0}{t_0}$$

where t_r is retention time of the drug and t_0 is column void volume time of a non-retained compound or reference (citric acid).

For those drugs with long retention times ($t_r > 60$ min), acetonitrile was added to the mobile phase. Back extrapolation of plots of $\log k'$ versus percent of acetonitrile allowed determination of capacity factor for an entirely aqueous phosphate buffer solution.

2.5. Data set and QSAR model building

The human intestinal absorption values for 52 drugs (%HIA) (Table 1) were collected from the literature and 76 molecular descriptors (topological, geometric and physicochemical descriptors) and solubility parameters were calculated for each drug using Molecular Modeling Pro Ver 5.1 (ChemSW Inc.).

Statistica TM (StatSoft) was used for building the QSAR. Multiple forward stepwise regression was used to select input variables among k' and molecular descriptors that best describe intestinal absorption (dependent variable or %HIA). The initial step was to build a model with a single independent variable and the dependent variable, and then the model was repeatedly altered by adding one independent variable at a time until the relationship was no longer improved.

Table 1
Measured capacity factors (k') and corresponding observed [29–33] and predicted intestinal absorption (IA (%)) of drugs

	k'	$1/k'$	Observed IA (%)	Predicted IA (%)
Acebutolol	12.71	0.08	89.50	81.03
Acetyl-L-carnitine	0.11	9.52	5.00	4.35
Amantadine	4.19	0.24	95.00	104.11
Aspirine	0.26	3.85	100.00	60.42
Amoxacillin	0.34	2.99	94.00	71.66
Atenolol	1.48	0.68	50.00	68.84
Benzylpenicillin	3.53	0.28	30.00	80.35
Betaxolol	11.57	0.09	90.00	84.20
Bretylium	1.34	0.75	18.00	17.22
Caffeine	2.29	0.44	100.00	97.96
Captopril	0.36	2.75	67.00	71.61
Cephalexin	0.75	1.34	98.00	68.71
Chloramphenicol	18.18	0.05	90.00	76.04
Chlorothiazide	7.24	0.14	13.00	58.32
Cimetidine	2.39	0.42	85.00	58.67
Ciprofloxacin	25.84	0.04	67.00	84.40
Desipramine	7.67	0.13	100.00	94.68
Diazepam	135.49	0.01	100.00	103.06
Enalapril	0.46	2.17	65.00	62.01
Furosemide	39.73	0.03	61.00	65.99
Hydrochlorothiazide	5.68	0.18	67.00	62.67
Hydrocortisone	61.94	0.02	91.00	89.53
Ibuprofen	72.53	0.01	100.00	97.41
Imipramine	73.20	0.01	95.00	111.03
Ketoprofen	68.71	0.01	100.00	98.31
Labetalol	118.77	0.01	95.00	84.80
L-Carnitine	0.07	15.15	5.00	−11.15
Mannitol	0.11	9.43	15.00	41.51
Metoprolol	7.43	0.13	95.00	88.53
Naproxen	43.32	0.02	99.00	103.01
Oxprenolol	18.24	0.05	90.00	88.55
Paracetamol	2.19	0.46	80.00	79.78
Phenytoin	40.37	0.02	90.00	76.63
Pindolol	11.35	0.09	90.00	79.61
Prednisolone	38.37	0.03	98.80	91.50
Progesterone	536.41	0.00	91.00	91.70
Propionyl-L-carnitin	0.21	4.85	5.00	19.15
Propranolol	117.76	0.01	90.00	89.51
Pseudoephedrine	2.16	0.46	100.00	89.26
Quinidine	56.35	0.02	80.00	90.46
Ranitidine	2.89	0.35	50.00	59.01
Ribavirin	0.11	9.25	33.00	38.34
Salicylic acid	3.54	0.28	100.00	88.01
Scopolamine	5.13	0.19	90.00	80.43
Sulfasalazine	89.17	0.01	65.00	49.83
Sulindac	27.40	0.04	90.00	95.38
Terbutaline	2.44	0.41	73.00	88.86
Timolol	6.98	0.14	90.00	84.43
Triamterene	27.14	0.04	100.00	85.23
Trimethoprim	10.77	0.09	97.00	86.62
Verapamil	64.20	0.02	90.00	86.32
Warfarin	132.65	0.01	98.00	97.44

3. Results and discussion

For most drugs, the predominant mechanism of oral absorption is via the transcellular route. It relies on the ability of the molecule to partition into and move across gastrointestinal epithelium membranes. The most important features of a drug that influence this partitioning are solubility, permeability and molecular size. Accordingly, the first step in the current study was to calculate bulk properties, solubility parameters and topological descriptors (Table 2) of the selected drugs and to measure the retention (capacity factor, k') of each drug on the IAM column as an indicator for the drug partitioning into cell membrane. The next step was to correlate calculated descriptors and experimentally measured capacity factor against %HIA. Since an equation containing an excessive number of independent variables can be too

Table 2
Calculated molecular descriptors

Bulk properties	Molecular mass, Van der Waals volume, surface area, molecular volume [34], molar volume [35], density, molecular length, width and depth
Solubility parameters	Octanol–water partition coefficient (fragment addition [36] and atom based log *P* [37]), molar refractivity (MR), *Q* log *P* [38], hydrogen bonding number, solubility parameter and 3D solubility parameters (dispersion, polarity and hydrogen bonding) (van Krevelen, and Hansen's methods), mean water of hydration [39], hydrophilic–lipophilic balance (molecular mass and volumetric HLB), hydrophilic surface area and percent hydrophilic surface area, polar surface area [40], surface tension, water solubility [41] (log *W*–log water solubility, g/L, [ppm]), log Sw (water solubility estimated from log Kow [42]), log Kow (log molar water solubility), log molar olive oil–gas partition coefficient [43]
Topological descriptors	Randic connectivity indices [44] (Chi 0–Chi 4), valence connectivity indices [45] (Chi V1–Chi V4), Kier's kappa shape indices [46] (kappa 1–3), difference indices (0–4), 3D Wiener number [47], chemically intuitive molecular indices (eigenvalue 1–14) [48]

cumbersome to use and is likely to be overparameterised, we utilised stepwise regression to refine the model and to select most important descriptors to generate regression equation.

Hydrophilic lipophilic balance (HLB) value, hydrophilic surface area (HSA), polar surface area (PSA) and dipole moment gave the best fit with R^2 value of coefficients being 0.91, 0.90, 0.82 and 0.82, respectively (Table 3). Stepwise regression method confirmed that in addition to solubility (solubility parameter, HLB, and HSA), polarity (dipole moment and PSA), lipophilicity (capacity factors (k')) and molecular size and shape (molecular width, length, and depth) can be successfully used to predict intestinal absorption (Table 4). The combination of all selected descriptors gave the correlation value of 0.83. Among these descriptors, HLB, capacity factor k' and solubility parameter were the most significant. The optimum QSAR equation for predicting intestinal drug absorption can be expressed as follows:

Human intestinal absorption (HIA%) = 114.4 − 4.89 (HLB) − 3.76 $(1/k')$ + 2.50 (solubility parameter) − 0.22 (PSA) + 1.32 (dipole moment) − 1.70 (molecular length) + 2.95 (HSA) − 2.18 (molecular width) − 2.00 (molecular depth).

Table 4 shows the statistical analysis of the established QSAR equation. The *p*-values obtained for all selected descriptors were less that 0.05, indicating that the relationship between these parameters and the HIA (%) was statistically significant at the 95% level. The coefficients (Table 3) were also significant ($p < 0.05$) at the same confidence level and standard error (S.E.) values were low.

Table 3
Multiple regression results

	Regression coefficient	S.E. of coefficients	*t* (42)	*p*-level	R^2
Intercept	114.37	45.96	2.49	0.02	
HLB	−4.89	1.27	−3.86	0.00	0.916
1/*k*	−3.76	1.56	−2.42	0.02	0.678
Solubility parameter	2.50	1.34	1.86	0.07	0.796
PSA	−0.22	0.17	−1.26	0.21	0.261
Dipole	1.32	0.92	1.43	0.16	0.820
Molecular length	−1.70	1.06	−1.60	0.12	0.496
HSA	2.95	1.86	1.59	0.12	0.900
Molecular width	−2.18	1.59	−1.37	0.18	0.470
Molecular depth	−2.00	1.71	−1.17	0.25	0.823

By comparing the magnitude of regression coefficients we can compare the relative contribution of each independent variable in the prediction of the dependent variable. R^2 = coefficient of determination, indicator of how well the model fits the data (an R^2 close to 1.0 indicates that we have accounted for almost all of the variability with the variables specified in the model).

Because a cell membrane is comprised of hydrophilic and lipophilic regions, a molecule that pass through a cell membrane through the transcellular pathway needs to penetrate both hydrophilic and hydrophobic environments. As a result, both hydrophilic and lipophilic properties of a drug should be taken into account when predicting drug permeability. In our study, the HLB value was found to be most important for predicting drug absorption and was negatively correlated to intestinal absorption. HLB is a measure of the proportion of a molecule's mass that is hydrophilic. As the HLB value increases, the hydrophilic character of a drug molecule increases. It is difficult for a drug molecule with mainly hydrophilic structure to penetrate the outer layer (phospholipid layer) of the cell membrane by transcellular diffusion. Thus, the intestinal absorption decreases as the HLB value increases.

Human intestinal absorption was reciprocally correlated to the negative value of the IAM capacity factor $(-1/k')$. Lipophilicity is one of the vital parameters commonly used to predict membrane permeability [10,11] and is approximately correlated to passive transport across cell membranes and the ability of a compound to partition a membrane [12]. Estimation of drug permeability using IAM chromatography alone (k') provided a good correlation ($R = 0.64$) with experimentally determined drug absorption (Fig. 1). Conventional ODS silica columns, can provide retention values of analytes solely on the basis of lipophilicity. On the other hand, IAM chromatography measures phospholipophilicity and more closely mimic the interaction of analytes with biological membranes, where a combination of hydrophobic interaction, ion pair interactions and hydrogen bonding interactions are possible [6]. The reciprocal correlation indicated the nonlinear relationship between k' and drug absorption. Many drug molecules contain one or more ionizable groups, and their lipophilicity changes with respect to pH resulting in nonlinear relationship with GIT absorption. This means that the correlation between IAM membrane permeability

Table 4
Summary of stepwise regression

	Step in	Multiple R	Multiple R^2 for each dependent variable	F to enter/remove	p-level
HLB	1	0.75	0.564	64.67	0.00
1/k	2	0.77	0.60	4.30	0.04
Solubility parameter	3	0.80	0.63	4.20	0.05
PSA	4	0.80	0.64	1.48	0.23
Dipole	5	0.81	0.65	1.19	0.28
Molecular length	6	0.81	0.66	0.39	0.53
HSA	7	0.81	0.66	0.68	0.41
Molecular width	8	0.82	0.67	1.48	0.23
Molecular depth	9	0.84	0.68	1.37	0.25

R = regression coefficient; F value for a variable indicates its statistical significance; it is a measure of the extent to which a variable makes a unique contribution to the prediction of group membership; p-level represents probability of error involved in accepting hypothesis that the differences between the parameter estimates are equal to zero, and hence, that they are of equal magnitude. Specifically, the coefficients are used to form linear combinations of parameter estimates, and these linear combinations are then tested against zero.

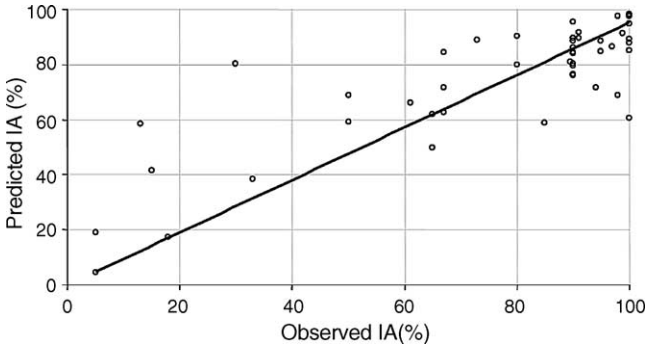

Fig. 1. Validation plot for QSAR model: predicted vs. observed IA (%) values.

and absorption is represented by two hyperbolic functions, a direct one for the lipophilic permeation, and inverse one for aqueous porous diffusion for compounds with low molecular mass that can use the aqueous pathway. The correlation was further improved by the addition of molecular descriptors representing molecular size and shape (with experimentally determined width, length and depth), solubility (solubility parameter, HLB, hydrophilic surface area) and polarity (dipole, polar surface area) (multiple $R = 0.84$).

Aqueous solubility [13,14] is another key parameter that is fundamental to allow good drug absorption. Unfortunately, the majority of new potential drug candidates are highly lipophilic, less soluble compounds with a higher number of hydrogen bond donors and acceptors and larger molecular size. Drugs need to be dissolved to be absorbed and there is a positive relationship between human drug absorption and aqueous solubility. Drugs with absorption problems are those with low solubility and low permeability. However, compound with high water solubility will have low lipophilicity and permeability. Thus, solubility profile of the drug should be considered with other factors when predicting intestinal absorption.

Small hydrophilic molecules pass through membrane via paracellular diffusion. Generally, molecular size and shape of molecules are relevant to the penetration through membranes. Molecular dimensions and shape are described by molecular width, length and depth. Compounds with larger molecular width, length and depth are not well absorbed from the intestinal epithelium due to the increase molecular size which will be limiting to pass through the paracellular pores.

Dipole moment and polar surface area are the measure of polarity of the molecule. Dipole moment describes the intramolecular electronic effect, which may be related to molecular reactivity [15]. The activity of a molecule increases as the dipole moment is increases. Drugs with high polarity are likely to be less absorbed from the small intestine. Polar surface area (PSA) also accounts for the steric shape of a molecule and has been found to be related to drug permeability [16]. PSA is a surface descriptor [17], defined as the part of the surface area of a molecule contributed by nitrogen, oxygen and connected hydrogen atoms. As such, it is clearly related to the capacity of a drug to form hydrogen bonds. Molecules with many H-bond donors and a large polar surface area yields low permeability values. It can be observed that, for molecules with large polar surface area, permeability increases with lipophilicity, while for molecules with small polar surface area, lipophilicity appears to have little effect on intestinal permeability. This observation can be traced to small water-soluble, lipid-insoluble molecules, as well for molecules with small polar surface areas. Such molecules are likely to exhibit good intestinal passage due to other mechanisms, besides passive transmembrane diffusion, through aqueous pores induced by hyperosmolality [18], or perhaps via paracellular diffusion [19]. As a consequence both polar surface area and hydrophilic surface area were included in the model, first with positive and second with negative correlation.

Developed equation predicted lower than expected HIA (%) values for atenolol, terbutaline, desipramine chlorothiazide and aspirin. Generally beta-blockers (atenolol) and beta-adrenergics (terbutaline) are absorbed from the GIT via passive diffusion. Therefore their absorption is not considered stereoselective [20]. However, some beta-blockers and

beta adrenergics may undergo an intestinal secretion process that may be modestly stereoselective, resulting in an apparent nonlinearity in the kinetics of the drug with increasing oral doses [21]. In the case of chlorothiazide, analysis of data at the four dosage levels of chlorothizide suggested that chlorothiazide absorption is possibly saturable [22]. Tricyclic antidepressants like desipramine also undergo dose-dependent kinetics [23,24]. The study shows that the rate of absorption is significantly faster for soluble aspirin than for solid aspirin regardless of fed or fasting state [25]. However, absorption of solid aspirin can be significantly affected by food and fasting state. On the other hand aspirin is a weak acid ($pK_a = 3.5$) which undergoes hydrolysis to form salicylic and acetic acid. The presence of carboxylic acid creates its own acidic environment and hydrolysis of the ester occurs quite rapidly. The product, salicylic acid, is even more acidic and catalyses a more rapid hydrolysis. It is very likely that hydrolytic instability of aspirin will affect the measured capacity factor.

Higher HIA (%) values were predicted for ciprofloxacillin, hydrochlorthiazide, triamteren, bretylium, ribavirin, enalaprilat. Cell membrane transport of β-lactam antibiotic is dose dependent [26]. Compared with the young volunteers, mean plasma concentrations of triamterene, hydrochlortiazide [27] are significantly higher in elderly patients. While plasma concentrations of these drugs are increased in correlation to age of subjects and duration of therapy, urine flow and renal Na^+ excretion are decreased at the same degree. With aging, total body water and lean body mass are reduced, and body fat is increased. The relative decrease in total body water and thus in sodium space leads to higher blood (and often tissue) concentrations of some water-soluble drugs. Increased fat increases the volume of distribution for lipophilic drugs and may increase their elimination half-lives. Bretylium [28] is antiarrhythmic agent, used for the management of serious and refractory ventricular tachyarrhythmias. It exhibits a complex pharmacokinetic profile which is poorly understood. The drug is poorly absorbed following oral administration, and its oral bioavailability is in the region of 18–23%. Ribavirin is rapidly and extensively absorbed after oral administration, followed by rapid distribution and prolonged elimination phases. Due to first-pass metabolism its absolute bioavailability is approximately 50%.

4. Conclusion

Experimentally measured retention (capacity factor, k') on immobilized artificial membrane at pH 5.4, together with molecular descriptors and solubility parameters, were correlated with intestinal absorption of drugs in humans. Given the good reproducibility of IAM chromatography and its short analysis time, experimentally measured capacity factors and calculated molecular descriptors can be used as a screening method for the selection of drug candidates in the early stage of drug discovery process.

The developed QSAR model does not require animal or human experiment data and could potentially provide useful information on intestinal drug absorption in humans. However, due to the difficulties in obtaining information on middle or low absorption drugs, the data used is biased towards well-absorbed drugs. Furthermore, the diversity of intestinal absorption data in the literature is another source of possible error while building the model. Finally, few compounds in the data set are subjected to various active transport mechanisms and the contribution of these active transport pathways to total intestinal absorption is not been fully understood. These factors all influent the accuracy of this model.

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